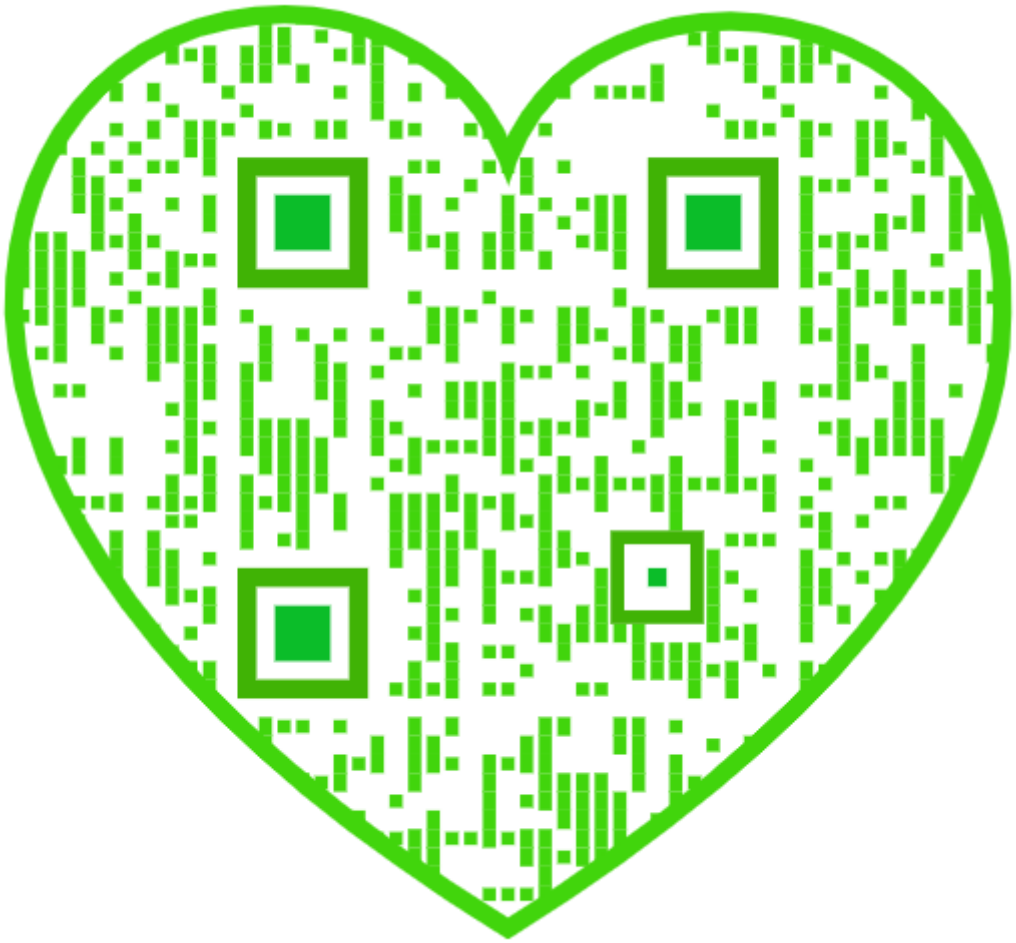

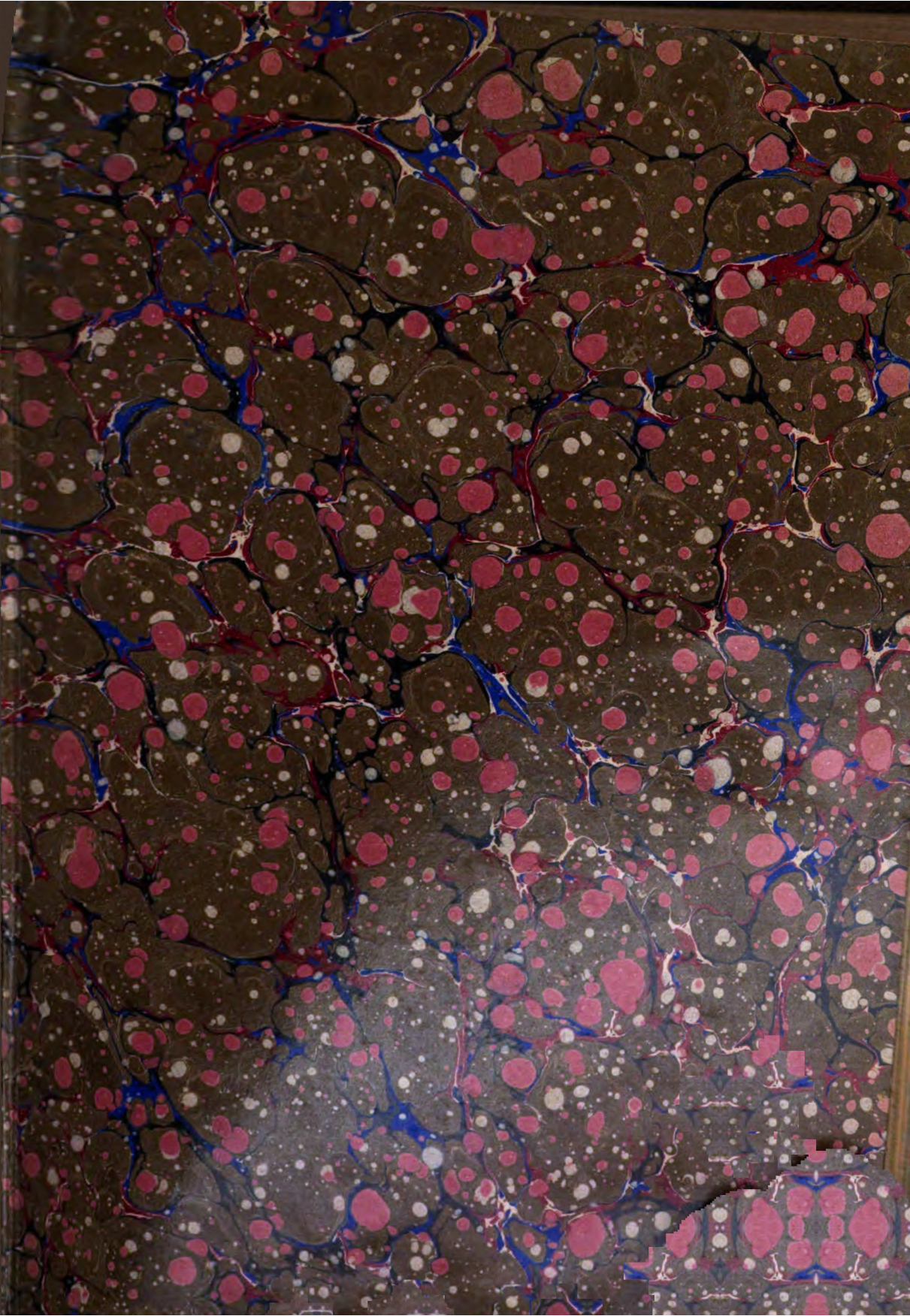

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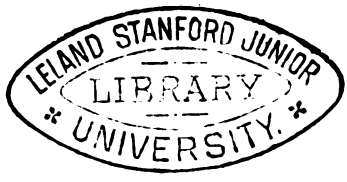

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ASSISTED BY OTHER BOTANISTS

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On the Genus Myeloxylon (Brong.).

BY

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Lecturer in Botany in the University of Cambridge.

With Plates I and II.

IN *Myeloxylon*[1] we have one of those genera of palaeozoic plants with which palaeobotanists have long been familiar, but as to whose botanical affinity the balance of opinion appears to be fairly equally divided. Numerous examples have been figured from English, German, and French localities, but none with tissues sufficiently well-preserved to admit of a complete examination of their anatomical characters. Without claiming for the specimens described in the present paper that they settle the botanical position of the genus, I hope to show that they afford further evidence in support of the view that *Myeloxylon* agrees more closely with recent Cycads than with Ferns. Before considering our new material in detail, it will be well to review the descriptions of previous writers and note the arguments advanced in support of the different opinions as to the relation of *Myeloxylon* to recent plants.

[1] I use this name because it has been adopted by Solms-Laubach in his 'Einleitung in die Paläophytologie,' as better known than the generic name of *Stenzelia*, although the latter has ten years' priority according to Goeppert and Stenzel (Die Medulloseae, p. 10 [Palaeontographica, vol. XXVIII. 1881]).

[Annals of Botany, Vol. VII. No. XXV. March, 1893.]

In Corda's great work, Flora der Vorwelt[1] (1845), a figure is given of a transverse section of a plant, from Radnitz in Bohemia, under the name of *Palmacites carbonigerus*. As the name implies, this was considered to be a Palm. In his description of the specimen Corda points out that much of the bundle-tissue has been destroyed, and the spaces filled with mineralizing material. In some places (Figs. 4 and 5, Pl. XX.) sclerenchymatous elements are shown in close connection with the vascular bundles. The tracheids are described as scalariform: no mention is made of mucilage-canals, but in Fig. 5 Corda has represented what we may in all probability regard as such. This species of *Palmacites* bears a strong resemblance to *Myeloxylon* (*Myelopteris*) *radiata*.

In 1832 Cotta[2] instituted three species of *Medullosa*; *M. elegans* is the only one with which we are at present concerned. This species is recorded from Rothliegende (Permian) rocks near Chemnitz and Kohren. In the Robert Brown collection in the British Museum is a specimen which was no doubt cut from the same piece as the section figured by Cotta in Pl. XII. Fig. 2; in this section a number of dark lines of crushed cells are seen in the parenchymatous ground-mass surrounding small 'islands' of mineralized tissue. This mode of fossilization is fairly common in *Myeloxylon* and well marked in some of the examples to be described later.

Brongniart[3], in his Tableau des genres de végétaux fossiles, changes the name *Medullosa* to *Myeloxylon*, and classes *Myeloxylon elegans* as a doubtful Monocotyledon. Goeppert[4] describes the hypodermal tissue of *Myeloxylon* as wood; he considers the dark lines in the ground-tissue as part of the natural structure of the plant and not as an accident of

[1] Corda, Beiträge zur Flora der Vorwelt, Pl. XIX. Fig. 2.

[2] Cotta, Die Dendrolithen, Pl. XII. Figs. 1 and 2.

[3] Brongniart, A., Tableau des genres de végétaux fossiles, considérés sous le point de vue de leur classification botanique et de leur distribution géologique (Dictionnaire universel d'Histoire Naturelle, vol. XIII. p. 59 [1849]).

[4] Goeppert, Die fossile Flora der Permischen Formation, p. 218 (Palaeontographica, XII. 1864–1865).

mineralization. The tracheids are described as scalariform and reticulate. In conclusion Goeppert refers *Myeloxylon elegans* to those plants which must be looked upon as prototypes or synthetic types; he recognises Ferns, Gymnosperms, and Monocotyledons as represented in certain structural details of this palaeozoic species.

Binney[1], in 1872, refers to *Medullosa elegans* of Cotta as the rachis of a Fern or of a nearly-allied plant.

In 1873[2] Williamson refers the same plant to the Marattiaceae: Schimper, on the other hand, classes *Myeloxylon* with the Cycads.

In 1876[3] Renault contributed an important paper in which a number of silicified specimens of *Myeloxylon* (*Myelopteris* of Renault, *Stenzelia* of Goeppert) from Autun and Saint Étienne are figured and described in detail.

Several of the French specimens are much larger than any found in English rocks. The vascular bundles, which, especially in the small petioles, are arranged in fairly concentric lines, Renault describes as consisting of large scalariform vessels with smaller barred and spiral tracheids: the vacant space, which occurs in each bundle next to the group of xylem-elements, is referred to as a gum-cavity. In Plate IV. Fig. 27, one of these spaces is shown containing several cells or 'tubes' with their walls considerably thickened; these are certainly not such elements as one would expect to find forming part of the phloëm which originally occupied the vacant spaces in each bundle. Probably these so-called gum-cells with thick walls have found their way into the cavity left by the decay of the phloëm. In a later work, Renault[4] makes no mention of the thick-walled cells, and speaks of the bast-part of the bundles as nearly always absent. Certain variations in the arrangement of the hypodermal prosenchyma have

[1] Binney, Proc. Lit. and Phil. Soc. Manchester, vol. XI.

[2] In a communication to the Brit. Assoc. meeting at Bradford.

[3] Renault, Recherches sur les végétaux silicifiés d'Autun—étude du genre *Myelopteris* (Mém. par divers savants; Acad. des Sci., Instit. de France, vol. XXVII [1876]).

[4] Cours de botanique fossile, vol. III. p. 162 (1883).

been made use of by Renault as a basis of classification: in one type, *Myelopteris Landriotii*, the fibrous bundles of the hypoderm are circular, elliptical, or reniform; in the second type, *M. radiata*, they are flattened and arranged as radiating bands of sclerenchymatous tissue. Our English specimens agree more closely with the *M. radiata* type. Whilst admitting a few points of difference between *Myelopteris* and recent Ferns, Renault concludes that its true position is with the Marattiaceae. One of the arguments considered by him of great weight in favour of this decision is the common association of *Alethopteris*-leaflets and *Myelopteris*-petioles; and in one case a rachis of *Alethopteris aquilina* is figured showing the same structure as that of *Myelopteris*. The force of this and other arguments will be considered at the conclusion of these notes. Before passing on to Williamson's description of *Myeloxylon*, I may briefly refer to a specimen in the British Museum collection[1] which throws a little more light on the structure of the vascular bundles. This specimen, unfortunately with no record of locality, came in all probability from one of the places from which Renault's silicified specimens were obtained: its general structure and mode of preservation strongly support this view. In Fig. 1, where I have represented one bundle and two canals, a number of thick-walled elements are clearly shown at *s* in contact with the xylem, and at *p* what we may probably consider as one of the protoxylem-tracheids. In the space next to the protoxylem are traces of torn and delicate tissue (*a*), no doubt remnants of phloëm: surrounding the phloëm half of the bundle is a sheath of small parenchymatous cells.

In his seventh memoir[2] on the Organization of the fossil plants of the Coal-measures, Prof. Williamson gives some account of the history of *Myelopteris* and describes a number of specimens from the English Coal-measures. In these

[1] For access to specimens in the British Museum my thanks are due to Mr. Carruthers.

[2] Williamson, On the organization of the fossil plants of the Coal-measures, Pt. VII. Phil. Trans. Royal Society, 1876.

fragments there are certain characters which suggest the possibility that they ought not to be included in the same genus as those described by Renault and others, or as the fragments which I have figured. In Williamson's specimens there are peculiarities in the structure of the bundles, and in the arrangement of the hypodermal tissues, which distinguish them from other described examples of *Myeloxylon*: at the same time there are several points of resemblance. The spaces in the bundles, originally described by Williamson as gum-canals and shrinkage-cavities, are now recognised by him as 'true phloëm-portions of the fibro-vascular bundles[1].' *Myeloxylon* is classed by him with the Ferns, and the points of agreement with the Marattiaceae are considered at some length. Some specimens, which Prof. Williamson has generously lent to me for examination, may, I hope, throw further light upon the botanical position of those to which I have briefly alluded.

Grand' Eury[2] has figured what he considers to be a piece of the epidermis of *Myeloxylon*, showing a number of stomata. He points out a general resemblance between this genus and *Angiopteris* as regards the arrangement of the vascular bundles, but recognises a difference in the shape of the individual bundles; in *Angiopteris* they are elongated concentrically, in *Myeloxylon* they are either circular or elongated radially.

We come next to an important paper by Schenk[3], published in 1882. An examination of Cotta's original specimens in Berlin led Schenk to the conclusion that *Myeloxylon* resembles Cycadean petioles more closely than the petioles of Marattiaceae. In this paper we find the collateral nature of the bundles insisted upon as a strong argument in favour of Cycadean affinities: many other characters, whose importance had not been previously recognised, are dealt with in detail.

[1] Letter from Prof. Williamson, June, 1890.

[2] Flore Carbonifère du département de la Loire et du centre de la France (Mém. prés. par divers savants à l'Acad. des Sci. de l'Instit. de France, vol. XXIV. 1877. Pl. XIII. Fig. 7).

[3] Schenk, Ueber *Medullosa elegans* (Engler, Bot. Jahrb., vol. III. p. 156, 1882).

Felix[1], in a more recent paper, agrees with Schenk as to the close resemblance between *Myeloxylon* and Cycads: he gives a figure of a specimen from Westphalia, and notes the occurrence of certain narrow cells in the parenchyma which he considers may explain those dark lines so often seen running through the ground-mass of *Myeloxylon*-petioles. Schenk and others prefer to regard these dark lines as accidents of preservation. Such specimens as I have examined lend no support to the suggestion of Felix. The question of the true nature of *Myeloxylon* has been fully and impartially discussed by Solms-Laubach[2] in his Einleitung in die Paläophytologie: he figures a single vascular bundle which shows more of the phloëm preserved than in specimens previously described. Solms adds in a footnote an important fact in connection with *Myeloxylon* and recent Cycads: he remarks, 'the latest observations have made it probable that the Myeloxyla are the leaf-stalks of Medullosae.' 'If this is established,' he adds, 'a new and important weight will be added to the scale on the side of Cycadeae.'

In a discussion on *Myeloxylon*, Schenk[3] refers to Williamson's specimens as probably more nearly related to Marattiaceae than are those of Renault. The same author[4], in a paper on *Medullosa* and *Tubicaulis*, speaks of the *Myeloxylon*-like branch of *Medullosa Leuckarti* referred to by Solms, and admits that the suggestion of Williamson and Renault as to the Fern-like nature of *Myeloxylon* may be correct, but at all events it must be looked upon as belonging to an extinct group.

Mr. Kidston[5] has figured a bundle of *Mycloxylon* from the

[1] Felix, Untersuchungen über den inneren Bau Westfälischer Carbon-Pflanzen (Abhandl. d. K. Preuss. geolog. Landesanstalt, Berlin, 1886).

[2] Solms-Laubach, Einleitung in die Paläophytologie, p. 165, 1857 (Fossil Botany, p. 161, 1891).

[3] Schenk, Die fossilen Pflanzenreste, p. 45 (Handbuch der Botanik, 1888).

[4] Schenk, Ueber *Medullosa*, Cotta, und *Tubicaulis*, Cotta (Abhandl. der Math. phys. Classe der K. Sächs. Gesells. der Wiss., vol. XV. p. 523. Leipzig, 1889).

[5] Kidston, R., On the fructification and internal structure of carboniferous Ferns in their relation to those of existing genera, with special reference to British palaeozoic species (Trans. Geol. Soc. Glasgow, vol. IX. Pt. 1), Pl. IV. Fig. 45.

Coal-measures of Oldham, in which the phloëm is partially preserved: the drawing is hardly sufficiently detailed to admit of a full description, but the specimen appears to agree very closely with that from the Binney collection which is described and figured in the present paper. Kidston compares *Myeloxylon* to *Ophioglossum vulgatum* and to Osmunda, and speaks of it as 'a typical marattiaceous fern-stem'[1].

Myeloxylon from the Millstone Grit.

It will be convenient to describe such specimens as I have examined under three heads: those to be considered first afford an addition to the short list of Millstone Grit plants[2]. These plant-fragments were found in a block of magnesian limestone, of Millstone Grit age, about five miles N.E. of Lancaster[3], by Mr. J. E. Marr. The block is represented in Fig. 2, somewhat less than natural size. In the upper part of the figure a group of rod-like structures is shown, weathered out from the rest of the rock, reminding one to some extent of the structure occasionally seen in limestones and known as 'stylolites.' Dr. Hinde, on seeing the specimen, pointed out the striking resemblance of these rods to the anchoring appendages of *Hyalostelia Smithii* (Young), one of the hexactinellid sponges[4].

The rods, on microscopical examination, were found to be isolated bundles of tissue weathered out from the rest of the organ of which they form a part. Among fossil plants from the English Coal-measures it is not uncommon to find specimens in which regularly defined areas in a stem or root have been mineralized with their minute structure perfectly preserved, whilst the rest of the tissue has been almost entirely destroyed before the infiltration of the fossilizing

[1] Kidston, loc. cit. p. 50.

[2] Etheridge, in his Catalogue of Palaeozoic fossils, mentions twenty-eight species of British Millstone Grit plants (Fossils of the British Islands, vol. I. Palaeozoic, 1888).

[3] Near Caton, Geol. Surv. Map (inch scale), quarter sheet 91 N. E.

[4] For description of this sponge, see Palaeontographical Society, vol. XLI. p. 158.

material [1]. Fig. 3 shows this partial mineralization of the tissues as seen in a transverse section cut parallel to the base on which the block rests in Fig. 2. In this section we have a number of isolated islands of tissue, in each of which the xylem-portions of the vascular bundles are well preserved, whilst the phloëm is always absent; and in the immediate neighbourhood of the bundles are a few layers of parenchyma, with here and there distinct canals. At the periphery of the section are seen darker patches which indicate the position of thick-walled hypodermal cells arranged in radiating bands and alternating with radial extensions of the parenchymatous tissue. In many of the groups of tissue seen in Fig. 3, the parenchyma surrounding the vascular bundles is limited by a distinct dark line; this is due to the brown walls of the compressed parenchyma which represent the extent of the mineralization of the tissues. Similar dark lines, cutting up the parenchyma into a kind of network, are shown in Cotta's figure of *Medullosa elegans*, and also in a figure of *Myelopteris* given by Renault: I have already alluded to such lines as probably accidents of fossilization in speaking of Felix's note on *Myeloxylon*. In Fig. 3 a second fragment is represented, smaller than the first and cut longitudinally. To describe in detail the larger transverse section, which measures 2·5 cm. by 2·2 cm.: in looking at the peripheral bundles, where the ground-tissue has not been destroyed, and their original position has therefore been retained, we notice that they are regularly arranged concentrically with the circumference of the section; towards the centre this concentric disposition is much less obvious. The individual bundles, when examined in detail, appear under various forms. In some cases we have a group of xylem almost in the centre of a space bordered by parenchymatous cells: this type is shown in Fig. 4, in which case the position of the xylem suggests a partially preserved concentric bundle, the space having been originally occupied by the more delicate tissue of the phloëm. In Fig. 5, we

[1] There is a striking example of this in a large stem of *Araucaroxylon* in the British Museum (Geological Department).

have another type of bundle, where the xylem-group, which is triangular in form, is still more or less central in position; at the apex of the triangle some smaller tracheids are seen which suggest protoxylem. In all the vascular bundles of this specimen the parenchymatous cells immediately surrounding the xylem and the phloëm-space are smaller than the ordinary cells of the ground-tissue.

In Fig. 6, a third bundle is represented in which the xylem has been very slightly displaced, and here again smaller elements indicate that the probable position of the protoxylem is on the side of the xylem opposite to the phloëm-space. Close to this bundle we have one of the fairly abundant canals (*c*) which are scattered through the parenchyma; bordering the canal-cavity are some tangentially elongated cells, such as we find in mucilage-canals of recent Cycads.

Another kind of bundle is shown in Fig. 7; the xylem is still attached to the parenchyma at one or two points, and, as usual, on the side next to the vacant space are seen tracheids of smaller diameter.

The *Parenchyma of the ground-mass* appears to be made up of cells fairly uniform in size, except in the immediate neighbourhood of the vascular bundles, where the cells are marked off from the rest by their smaller size, and form a kind of indistinct sheath. Traversing the parenchyma are several canals: these are especially abundant towards the periphery of the section, but this may be due to the fact that the parenchyma towards the centre is much less perfectly preserved.

In Fig. 8, a patch of *hypodermal tissue* is shown; the preservation is by no means perfect, but sufficiently good to enable us to recognise in the alternating groups of parenchyma and sclerenchyma the same type of structure so clearly seen in Renault's figures of *Myeloxylon radiata*.

In this transverse section (Fig. 3) I have not made out with certainty any groups of thick-walled elements, either in close proximity to the xylem-groups or in the ground-tissue, but there are indications that such elements were here and there

present close to the bundles. The longitudinal sections of the same specimen throw little light upon structural details: the tracheids appear to be scalariform, reticulate, and spiral.

The smaller fragment seen in longitudinal section in Fig. 3 shows, in transverse section, certain characters which are apparently absent in the larger section. In Fig. 9 one of the best bundles is figured; the structure is clearly collateral, and the xylem has not been displaced as in the bundles of the larger fragment. Here also we find smaller tracheids on the side of the phloëm-space, which I am inclined to regard as protoxylem. Surrounding the bundles are smaller parenchymatous cells, with here and there thick-walled mechanical elements. In the same figure is shown a canal with a dark-coloured substance in its cavity, probably the remains of the original secretion. Several of these canals are represented in Fig. 10, the narrow cells surrounding the cavities being well shown; in this section we see indications of the characteristic radial groups of hypodermal tissue. Some of the thick-walled hypodermal cells are seen in longitudinal section in Fig. 11 at *a*; next to these are thin-walled and less elongated parenchymatous cells: at *a'* the tissue is badly preserved, but probably we have here the remains of a group of thick-walled hypodermal cells.

Passing farther along the section, we come to the parenchyma of the ground-tissue, the cells of which are somewhat elongated and arranged in rows. In the canal, cut longitudinally, the contents appear to be divided up into detached pieces by thin transverse septa: an examination of these canals under a higher power led me to doubt the existence of the septa and to attribute the present form of the contents to shrinkage. By comparing the transverse and longitudinal sections of the canals we see how the contents have contracted both transversely and longitudinally.

Part of a canal is represented in Fig. 12 under a higher power; in this figure the darker lines *bb* correspond to the walls *b* next to the canal-cavity in Fig. 10: the limiting lines *cc* of the dark-coloured contents in Fig. 12 are seen at *c* in Fig. 10.

In Fig. 2 at $\frac{1}{x}$ may be traced the elliptical outline of a section, cut obliquely: this is another fragment of *Myeloxylon*, in which the bundles have suffered less displacement than in the larger section, and the state of preservation is on the whole better. Thick-walled cells occur on the xylem-side of some of the bundles; and the tracheids appear to be reticulate and scalariform.

In this fragment we have a connecting link between the larger and smaller specimens, and an additional argument in favour of referring all the pieces preserved in the block shown in Fig. 2 to the same plant. From the above descriptions it is clear that there are a few points of difference between the large and small specimens. In the smaller fragment the bundles are more distinctly collateral; there are thick-walled cells, sometimes in a continuous row, on the xylem-side[1]; the canals are more numerous and larger than in the section figured in Fig. 3: but, in spite of these differences, we may include both specimens in the genus *Myeloxylon* and in the type *radiata* as instituted by Renault. Although many of the xylem-groups of the larger specimen occupy a position suggestive of concentric bundles, there are others, whose preservation is more perfect, which point with equal force to collateral bundles; and, on the whole, it seems reasonable to conclude that the apparently concentric structure is the result of the xylem-tracheids becoming detached from the surrounding parenchyma: originally, we may assume, the bundles were collateral. We shall find in nearly all specimens of *Myeloxylon* there are certain peculiarities; but, in dealing with such broken fragments of petioles, it is better to err on the side of including too many varieties under one general type than to attempt to define new species upon insufficient data. We are ignorant as to how far the structures of a *Myeloxylon*-axis of higher order differs from that of a lower

[1] As already remarked, similar mechanical elements are indicated in the large fragment: no doubt the worse state of preservation of this specimen is to some extent responsible for apparent differences between its structure and that of the smaller and more perfectly preserved fragment.

order; that branching does occur we know from the figures and descriptions of Renault[1]. Slight differences in structure may in some cases be the result of differences in fossilization; and further, as Thomae[2] has pointed out, in speaking of fern-petioles, it is hopeless to attempt to determine species by trusting to the anatomical details of detached fragments. The various specimens, therefore, described in these notes are referred to *Myeloxylon radiata* because of the possession of important characters in common. It is quite possible that further additions to our knowledge will render advisable the creation of additional species.

Specimens from Prof. Williamson's Collection.

One of the slides[3] lent to me by Prof. Williamson shows very clearly the essential characters of *Myeloxylon*: these characters are much more clearly defined in this specimen than in the other examples described by Williamson to which I have previously referred. In this section, prepared from an Oldham specimen, the collateral bundles are very distinct, but, as generally happens, the phloëm has not been preserved: thick-walled mechanical elements form an almost continuous ring round the xylem-groups. Another specimen of *Myeloxylon* from Oldham, in the Binney collection, agrees closely with Prof. Williamson's, but surpasses it in preservation; this will therefore be described at greater length.

Specimen from the Binney Collection.

In this collection, now in the Woodwardian Museum, Cambridge[4], I have found two slides of *Myeloxylon*, the one a transverse, the other a longitudinal section, which throw some fresh light on the nature of the vascular bundles.

In the transverse section, which measures 3 cm. in its longest part and 1·7 in its broadest part, the bundles have much the

[1] Renault, Etude du genre *Myelopteris*, Pl. V. Fig. 42.
[2] Thomae, Die Blattstiele der Farne (Pringsh. Jahrb. XVII. 1886, p. 158).
[3] Number 286 in Prof. Williamson's Catalogue.
[4] For the loan of this specimen I am indebted to Prof. McKenny Hughes.

same disposition as in the large specimen from the Millstone Grit; those near the periphery form a fairly distinct ring, whilst the others appear to have no definite arrangement. The hypoderm is of the normal type of *Myeloxylon radiata*, and need not be further considered. Canals are numerous and possess characters such as have already been described. Many of the bundles are of the type shown in Fig. 13; here we find, for the first time, not only the xylem but also the phloëm intact. In this particular bundle the smaller proto-xylem-elements are not quite so clearly marked as in most of the bundles, but the phloëm is preserved with exceptional distinctness. In Fig. 14 a second bundle is figured; here the phloëm is less perfectly preserved, but the small elements of the protoxylem are much more distinct: the sheath of small parenchymatous cells is clearly shown. In Fig. 15 we have another form of bundle, the xylem being in the form of an oblong group of tracheids, and the phloëm in two distinct groups on the same side of the xylem. In all these figures thickened mechanical elements are noticed on the xylem-side, and the presence of these is a constant character in the specimen. The striking resemblance of both these forms of vascular bundle to those of recent Cycadean petioles will be apparent when we sum up the arguments bearing upon the question of botanical affinity. In the longitudinal section of the same specimen we find, in two of the bundles at least, undoubted confirmation of what has previously been assumed but not actually demonstrated, namely, the occurrence of protoxylem or spiral tracheids on the phloëm-side of the xylem. Fig. 16 represents one of the bundles where we have large reticulate and scalariform tracheids in contact, on one side, with the parenchyma of the ground-tissue, and, on the other, separated from the less perfectly preserved phloëm by distinct spiral tracheids of small diameter. The phloëm shown at *P* is not sufficiently well preserved to enable us to describe its structure in detail; it consists of elongated, narrow, and broken elements, whose delicate walls have naturally suffered considerably during fossilization. At *GG* are seen

some cells of the ground-tissue. In Fig. 17 another bundle is represented in longitudinal section: the phloëm-elements are imperfectly preserved at *P*; next to them is a well-marked spiral tracheid, and following this what appears to be a second spiral element with larger scalariform and reticulate tracheids.

Having described at some length these specimens referred collectively to *Myeloxylon*, it only remains to consider briefly such facts as may help us in attempting to assign this palaeozoic fossil to its natural position in the plant-kingdom.

We may summarise, and briefly discuss, the arguments advanced by those who have referred *Myeloxylon* to the Marattiaceae.

1. *The association of* Alethopteris-*leaflets with* Myeloxylon, *and a close agreement in their anatomical characters.*

The figure which Renault gives in support of his statement that *Alethopteris*-pinnules have been found attached to a rachis having the structure of *Myeloxylon* is scarcely conclusive: so far as one can judge from the section represented [1], it is by no means obvious that the structure is the same as that of *Myeloxylon*. If, however, it is found that *Alethopteris* undoubtedly possesses this type of structure, we have no proof that in transferring it from the Ferns to the Cycads we shall be assigning it to a position to which it has no claim [2].

2. *The branching habit of* Myeloxylon.

Renault has given proof of this in his figures and descriptions of French specimens. Although *Bowenia*, as Solms reminds us, is the only recent Cycad in which the frond is branched, yet this solitary instance in living Cycads is not insufficient when we are dealing with plants of palaeozoic times. In such specimens as I have examined, no signs of branching have been detected.

3. *The vascular bundles, &c.*

It has been pointed out that *Myeloxylon* agrees in its bundles

[1] Renault, Cours de bot. foss. vol. III. Pl. 27, Fig. 12.

[2] Since this was written I have found some specimens in the Binney collection which. it is hoped, will afford further evidence in reference to *Myeloxylon*-pinnules.

and canals with *Marattia* and *Angiopteris*. The general arrangement of the bundles, as seen in transverse sections of *Angiopteris*-petioles, closely resembles that which we find in *Myeloxylon*; but this agreement ceases when the individual bundles are examined: in the one case we have a typical concentric, in the other a typical collateral bundle. In *Angiopteris* the tracheids do not show any very close resemblance to those of the fossil petiole; the reticulate thickening in the latter is characteristic, and differs from the type of tracheid characteristic of Ferns. As regards the presence of mucilage- or gum-canals in *Marattiaceae* and *Myeloxylon*, we do not find in the former those large canals, bordered by tangentially elongated cells, such as are constantly found in the fossil specimens, and which exactly correspond to the canals of Cycadean petioles.

4. *Hypodermal tissues.*

It is true that we find in *Angiopteris* a subepidermal band of mechanical tissue, but this is usually continuous and not interrupted by radiating groups of parenchyma.

Renault quotes *Angiopteris Brongniartiana*, and *A. Teismanniana* as examples of recent Ferns in which we have a more marked separation of thick- and thin-walled tissues; but, on the other hand, we can point to several species of Cycads where the hypoderm is of the same type as in *Myeloxylon*.

We will next consider the Cycadean affinities of *Myeloxylon*, some of which have been previously dealt with by Schenk.

1. *The collateral nature of the bundles, and the position of the protoxylem.*

This character, so clearly marked in well-preserved specimens, has been passed over by Renault and other writers. It is true, as Solms points out, that this structure is by no means unknown among recent Ferns: in addition to the collateral bundles in the petioles of *Ophioglossum* and *Osmunda*, Haberlandt[1] has shown that this is a common type in the

[1] Haberlandt, Ueber collaterale Gefässbündel im Laube der Farne (Sitzungsberichte der Math.-Naturwiss. Classe der K. Akad. der Wiss. vol. XXXII. p. 121, Abth. I, 1881. Vienna).

weaker subdivisions of the petioles of many genera. Granting, however, that this arrangement of xylem and phloëm may have been common in palaeozoic fern-petioles, we have still the important fact to remember that in recent Ferns *the protoxylem is not on the side of the xylem next to the phloëm: in* Myeloxylon *and recent Cycads it is so situated.*

2. *Nature of the tracheids.*

I have referred to the tracheids of *Myeloxylon* as reticulate, scalariform, and spiral. In Fig. 18 is represented part of an oblique section of the xylem of a specimen of *Myeloxylon* from Robert Brown's collection in the botanical department of the British Museum. This shows fairly well the character of some of the tracheids: at *p* we see what may possibly be the remains of spiral thickenings of protoxylem. In no case have I made out true bordered-pits, such as we find in recent Cycads, but it would be rash to attach any great weight to negative evidence in dealing with the minute structure of fossil tracheids. In spite of the absence of bordered-pits in *Myeloxylon*, there are various points of resemblance between its xylem-elements and those of Cycads. In some respects, for example in their reticulate structure, the tracheids of the fossil specimens do not correspond very closely either with Ferns or Cycads.

3. *Arrangement and form of the vascular bundles.*

The disposition of the bundles, as shown in transverse section, is in many recent Cycadean petioles very similar to that in *Myeloxylon*: in Ferns, on the contrary, the bundles are usually arranged in more distinctly concentric lines, such as we find in *Angiopteris*. As we have seen, the common form of the bundle in *Myeloxylon* is that of Fig. 13; this corresponds very closely with the shape of recent Cycadean bundles. In some specimens of *Myeloxylon* there are other bundles than the usual oval type, and these are also repeated in some recent species. If we compare Fig. 15, drawn from Binney's specimen, with the bundle of *Cycas Thouarsii* sketched in outline in Fig. 19, we see at once a close corre-

spondence[1]. Other forms of *Myeloxylon*-bundles might be quoted which find striking parallels in *Dioon edule, Ceratozamia, Cycas*, &c.

The fusion of two or more bundles in living Cycads has been referred to by Vetters[2] and also by De Bary[3]: in the fossil petioles similar instances of fusion are found.

4. *Hypoderm, subepidermal parenchyma, &c.*

In well-preserved examples of *Myeloxylon*, e.g. Fig. 41, Plate V in Renault's paper, there are a few layers of parenchyma immediately below the epidermis. Similar subepidermal parenchyma occurs in *Angiopteris*, as some writers have pointed out; but in petioles of Cycads it is also present. In many specimens of *Myeloxylon* this parenchyma has been destroyed, e.g. in Fig. 8, but in a small section, which occurs on the same slide as the larger Binney specimen, this tissue is clearly shown. The canals of recent Cycads and the fossil petioles are practically identical.

The hypodermal tissue of *Myeloxylon* closely corresponds with that of *Encephalartos horridus*, *Macrozamia Hopei*, and other species. In some of Renault's specimens we see groups of thick-walled fibres in the ground-tissue; Schenk refers to the absence of such in recent Cycads, but, as De Bary has remarked, groups of thick-walled fibres occur in various Cycadean petioles.

In the bundles of Cycads we find a few xylem-tracheids among the delicate phloëm-elements ('bois centrifuge'); these have not been detected in the fossil bundles, as Solms has already noted.

These tracheids are always much smaller than the other elements of the xylem, and, even in recent specimens, cannot in all cases be readily seen; it is not improbable that such might fail to be preserved, or, if preserved, to be distinguished from the surrounding phloëm.

[1] Similar bundles occur in *Stangeria paradoxa*, *Zamia muricata*, &c.

[2] Vetters, Die Blattstiele der Cycadeen (Inaug. Dissert., Leipzig, 1884).

[3] De Bary, Comparative Anatomy of the vegetative organs of the Phanerogams and Ferns, p. 337 (1884).

Finally, there is the connection between *Myeloxylon* and *Medullosa* referred to by Solms and Schenk. The former considers it an additional argument in favour of classing *Myeloxylon* with the Cycads; the latter, in his paper on *Medullosa* and *Tubicaulis*, refers to the attachment of *Myeloxylon* to *Medullosa Leuckarti*, but considers the latter more closely related to the Ferns than to Cycads. This fact has as yet been simply recorded without any full description.

I have endeavoured to state the more important arguments which have been made use of by the advocates of the two opinions as to the botanical position of the plant we have been considering. The conclusion to which I have been led by examining several specimens of *Myeloxylon*, and comparing them with numerous Cycadean and Fern petioles, is that they undoubtedly approach more nearly to the Cycadeae than to the Ferns: it has been suggested[1] that we may refer *Myeloxylon* to a position intermediate between Ferns and Cycads. The few points of difference which distinguish the fossil fragments from the petioles of recent Cycads are, in my opinion, far outweighed by the close parallelism in more essential characters, and it seems reasonable to conclude that *Myeloxylon* should be looked upon as an extinct genus, not exactly corresponding to any recent family of plants, but one which comes very near to the Cycadeae in anatomical structure, *and probably occupies a position between Cycads and Ferns, but nearer to the former than to the latter.*

[1] Solms-Laubach, loc. cit. (Eng. edit. p. 163).

EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Mr. Seward's paper on *Myeloxylon*.

(When not otherwise stated the figures are drawn with objective A, Zeiss.)

Fig. 1. Transverse section of a vascular bundle and two canals. *a* = remains of phloëm. *s* = sclerenchymatous cells. *p* = position of protoxylem.

Fig. 2. Block of Limestone (Millstone Grit) with weathered 'petiole' of *Myeloxylon*. On the smooth surface at $\frac{1}{x}$ is faintly indicated an oblique section of a smaller fragment. Actual size of specimen 10 cm. long and 10 cm. wide.

Fig. 3. Transverse section, rather less than natural size, of the larger petiole shown in Fig. 2, and longitudinal section of a smaller fragment. The white patches in the matrix outside the plant are sections of *Goniatites*.

(For the photographs from which Figs. 2 and 3 are taken, I am indebted to Mr. Barber.)

Fig. 4. Section of one of the vascular bundles of the specimen represented in transverse section in Fig. 3. The xylem has been displaced.

Fig. 5. Another bundle from the same specimen. *p* = protoxylem.

Fig. 6. " " " *c* = canal.

Fig. 7. " " " At *a* the xylem is seen still attached to the parenchyma of the ground-tissue.

Fig. 8. Piece of hypodermal tissue; in the large transverse section of Fig. 3 this appears as a black patch.

Fig. 9. Transverse section of a vascular bundle from a smaller fragment of the Millstone Grit *Myeloxylon*. *p* = protoxylem. *s*—*s* = sclerenchymatous cells. *c* = canal.

Fig. 10. A piece of the hypoderm and ground-tissue, with canals, from the same specimen as Fig. 9. *b* = tangentially elongated 'epithelial cells' of the canal. *c* = limiting lines of canal-contents. *h*—*h* = remnants of thick-walled hypodermal tissue.

Fig. 11. Longitudinal section of the same specimen as Fig. 10. *a* and *a'* = thick-walled cells of the hypoderm.

Fig. 12. Longitudinal section of a canal from the same specimen, showing the dark-coloured contents, &c. Letters same as in Fig. 10. (Objective D, Zeiss.)

Fig. 13. A vascular bundle from the Binney specimen, showing well-preserved phloëm.

Fig. 14. Another bundle from the same specimen. *p* = protoxylem. *s*—*s* = indistinct sclerenchymatous cells.

Fig. 15. This bundle shows two patches of phloëm, the result of fusion of two bundles.

Fig. 16. Longitudinal section of a vascular bundle from the same specimen as Figs. 14 and 15. *G* = ground-tissue. *P* = phloëm. *S* = spiral tracheid (protoxylem). (Objective D, Zeiss.)

Fig. 17. Another bundle from the same specimen. Letters as in Fig. 16. (Objective D, Zeiss.)

Fig. 18. Oblique section of xylem-elements in a specimen in the Botanical Department of the British Museum. *p* ? protoxylem. *a* = reticulate tracheid. (Objective D, Zeiss.)

Fig. 19. Outline of a bundle of *Cycas Thouarsii*, to compare with Fig. 15. *X* = xylem. *P* = phloëm.

Figs. 1 and 18 are from specimens in the British Museum.

Figs. 2–12 are from Millstone Grit specimens in the Woodwardian Museum, Cambridge, and in my own collection.

Figs. 13–17 are from the Binney specimen in the Woodwardian Museum, from the Oldham Coal-measures.

Fig. 1.

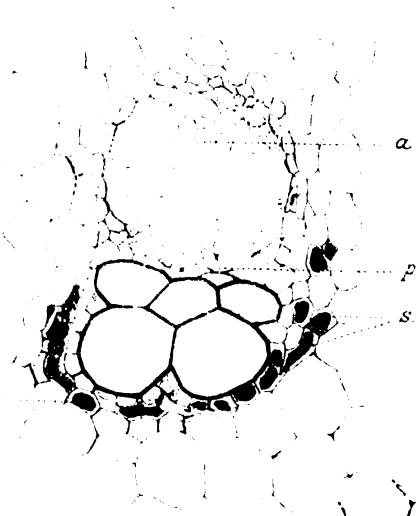
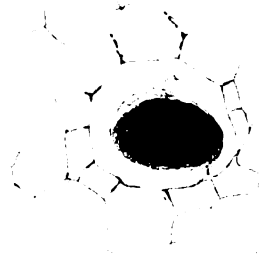


Fig. 2.



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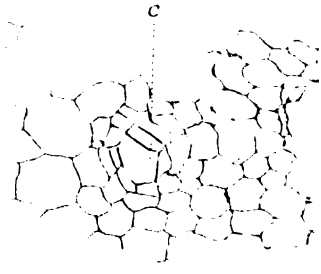

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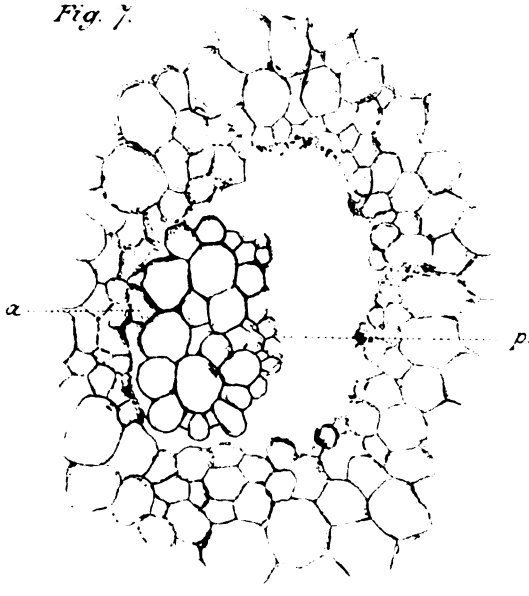

Fig. 8.

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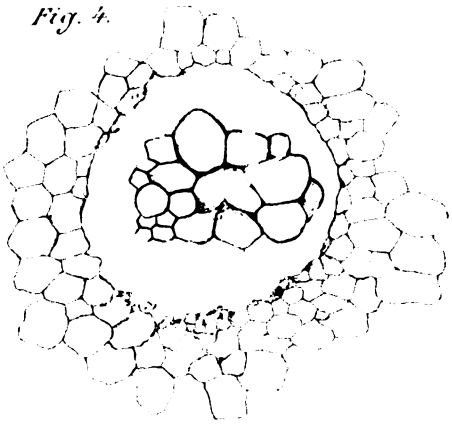
Fig. 3.

Fig. 4.



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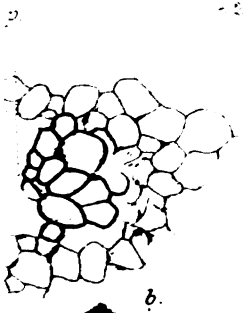

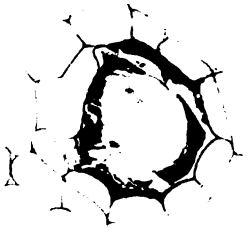

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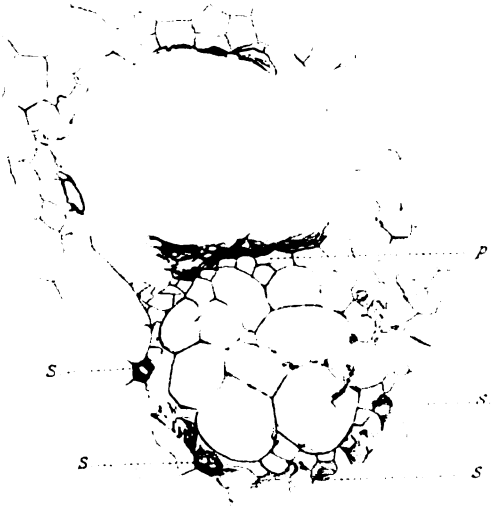

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Fig. 10.

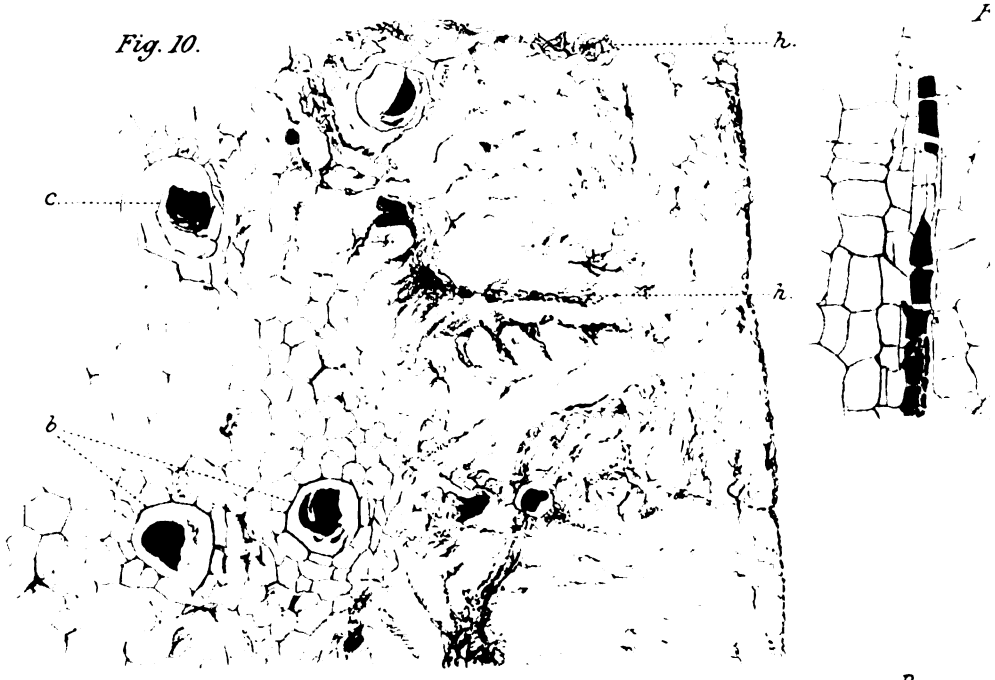
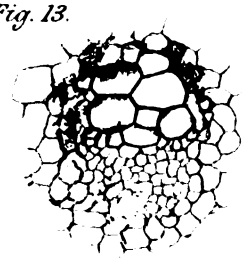


Fig. 13.



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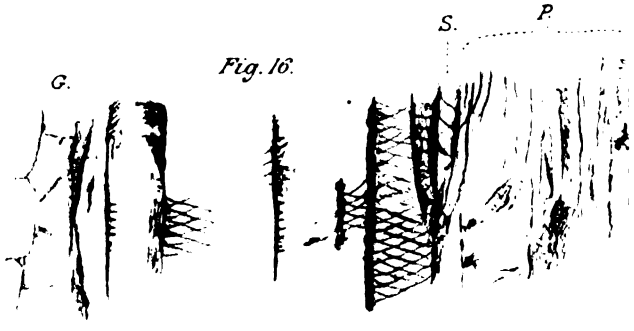

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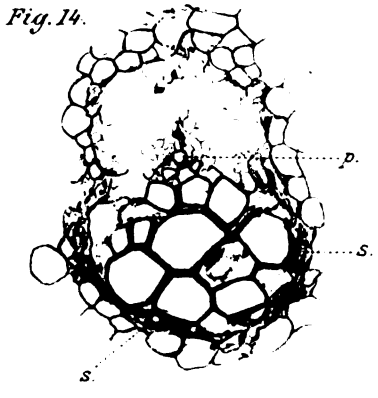

Fig. 17.

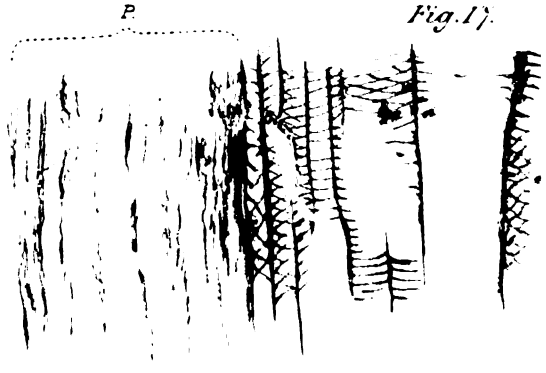

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Fig. 12.

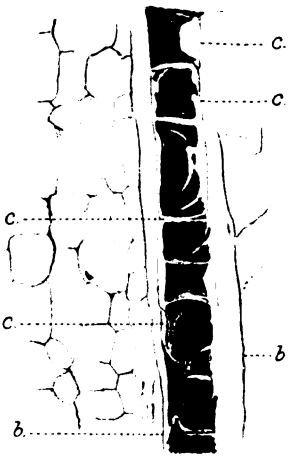


Fig. 15.

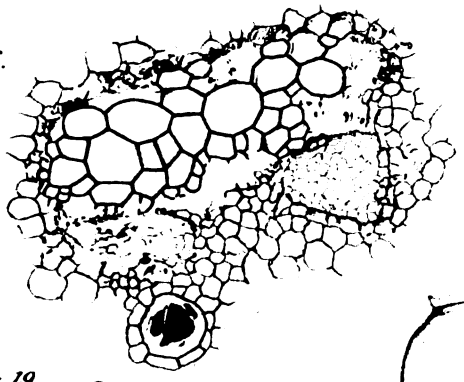
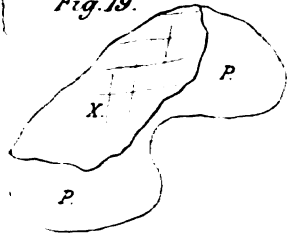
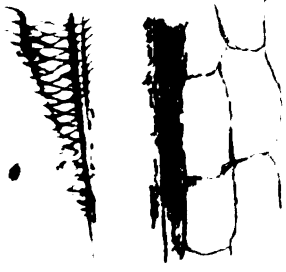


Fig. 19.





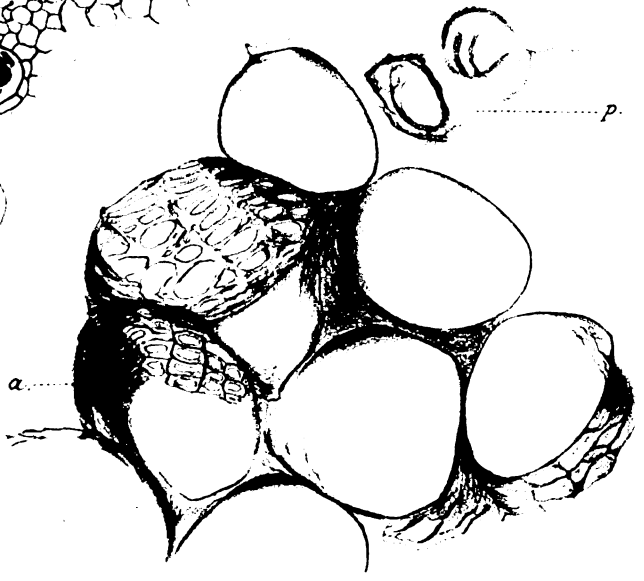


Fig. 18.

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On the Secondary Tissues in Certain Monocotyledons.

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With Plates III, IV, and V.

THE present paper treats of three distinct questions, relating to the general subject indicated by the title :—

1. The development of the secondary tracheides in *Yucca* and *Dracaena*.

2. The secondary growth in thickness of the roots of *Dracaena*.

3. The secondary growth in thickness of *Aristea corymbosa*, Benth. (N. O. Irideae).

No prefatory remarks to the whole paper are necessary, as a general knowledge of the monocotyledonous type of cambial growth is assumed.

I. The Development of the Secondary Tracheides in Yucca and Dracaena.

For the last seven years a controversy has been carried on as to the nature of the water-conducting elements in the secondary wood of *Dracaena*, and other Monocotyledons with a similar mode of growth. Up to 1886 it had been generally

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assumed that these structures are tracheides, each arising by the growth of a single cell, without any fusion of cells taking place[1]. But the first who endeavoured to establish this opinion on a firm basis was Krabbe, in his remarkable work on sliding-growth[2]. Although he states that he directly observed the young tracheides in process of elongation, he relies chiefly on the comparison of transverse sections, and on careful counting of the various elements of the secondary bundle at different stages of development. He established accurately the exact amount of growth in length of each cell which must take place; in *Dracaena Draco*, for example, he showed that the mature tracheide is, on the average, no less than thirty-eight times as long as the 'procambial[3]' cell from which it developes. As, in this case, the average number of tracheides in the transverse section of a mature bundle happens to be also thirty-eight, the striking conclusion follows, that as a rule the entire system of tracheides of each bundle arises from a single series of cells, so far as this species is concerned. The predominant share taken by sliding-growth in all such cases of tissue-development, is obvious.

A totally different account of the development was however given, almost simultaneously with Krabbe's work, by Kny[4].

[1] See, for example, De Bary, Vergleichende Anatomie der Phanerogamen u. Farne, 1877, p. 638, English Edition, p. 620: Strasburger, Das botanische Practicum, 1st edit. 1884, p. 127.

[2] Krabbe, Das gleitende Wachsthum bei der Gewebebildung der Gefässpflanzen, Berlin, 1886. Reviewed in Annals of Botany, vol. ii. p. 127.

[3] The word *procambium*, introduced by Sachs (see his Text-book, 2nd English ed. p. 110), means the strand of *primary* meristem from which a vascular bundle arises. Its name implies that it is a structure prior to the cambium, in cases where the latter is present. Hence procambium appears a very inappropiate name for a tissue which is itself produced from a *secondary* meristem, as is the case with the young bundles formed from the cambium in *Dracaena*, &c. The term *desmogen*, however (used by Russow as synonymous with procambium, see his Vergl. Untersuchungen, 1872, p. 178) may we think well be applied here. We propose to speak then of *secondary desmogen* to indicate the strand of young tissue from which a secondary bundle developes, in the Monocotyledons under consideration.

[4] Botanische Wandtafeln, Text, VII, 1886, and Beitrag z. Entwickelungsgeschichte der 'Tracheiden,' Berichte d. deutsch. bot. Gesellschaft, Bd. IV. p. 267, 1886. The former appeared just before, the latter just after, Krabbe's publication.

He endeavoured to show that the so-called tracheides are no tracheides at all, but short vessels produced by the fusion of longitudinal series of cells. A certain amount of sliding-growth at the ends of the vessels was however recognized. The stages of cell-fusion were not only described, but figured, by Kny. One of us (D. H. Scott) made some observations, which appeared at the time to confirm Kny's conclusions [1], which were further accepted by Strasburger, in the second edition of his Botanisches Practicum [2]. Independent evidence for the origin of these elements by cell-fusion was next brought forward in a paper by Mdlle. Hedwig Lovèn [3]. This observer also claims to have followed the stages of cell-fusion. Stress is further laid on the number of cells found in the transverse section of the bundle while in the merismatic condition. The number was found to be higher than that required by Krabbe's theory. Occasional, though very rare, traces of transverse walls in mature tracheides were also described.

So far there appeared to be a balance of evidence in favour of the origin of these tracheae by cell-fusion. In 1889, however, an elaborate paper appeared, by Röseler [4], containing the most complete account of the subject hitherto published, and bringing forward weighty arguments in proof of the development of these elements by the growth in length of single cells. Röseler attacked the question from every side, but his most convincing evidence was obtained by means of maceration. In a species of *Yucca* which he investigated, the average length of the mature tracheide is 3 mm.; and that of a cell of the secondary desmogen is 0·1 mm. Hence, on the average each desmogen-cell which becomes a tracheide must grow to thirty times its original length if no cell-fusion takes place.

[1] Annals of Botany, iv. p. 157, 1889.

[2] l. c. p. 125, 1887.

[3] Om Utvecklingen af de sekundäre Kärlknippena hos *Dracaena* och *Yucca*; Bihang till Kongl. Svenska Vetenskaps-Akad. Handlingar, vol. xiii. 1887. For a translation of parts of the Swedish text we are indebted to the kindness of Mr. L. A. Boodle, F.L.S.

[4] P. Röseler, Das Dickenwachsthum u. die Entwicklungsgeschichte der secundären Gefässbündel bei den baumartigen Lilien; Pringsheim's Jahrbuch., Bd. XX, 1889, p. 292.

If this be true, we ought to find, on macerating a young bundle, tracheides at all stages of elongation, and each of them would probably contain a single nucleus only. Röseler succeeded in demonstrating that these conditions are realized. He figures young thin-walled tracheides, isolated by maceration, measuring 0·4 mm., 0·84 mm., and 1·8 mm. in length, i. e. about four times, eight times, and eighteen times respectively, the length of the original desmogen-cell. Each contained a single conspicuous nucleus[1]. These observations were in reality decisive, but the effect of Röseler's work was somewhat enfeebled by the weakness of some of his other arguments. Thus his elaborate countings of the elements in transverse sections led to no definite result, but might seem rather to favour a mixed origin, by cell-fusion with subsequent elongation[2]. He also failed to obtain any satisfactory evidence from longitudinal sections, in consequence of manipulative difficulties[3]. In spite, therefore, of the great merits of his paper, we cannot wonder that it failed to settle the question. Röseler's work was reviewed by Wieler, in the Botanische Zeitung[4], with great and indeed unjustifiable severity. Wieler, however, contributed nothing himself to the decision of the points in dispute.

In 1891 Strasburger published his great work on the vascular tissues, and in this he withdraws his former view, and declares in favour of the origin of the tracheides by direct elongation of the short cells derived from the cambium[5]. He directly observed the young tracheides in sections, and confirms Röseler's statement that they are uninucleate. The observations of Röseler and Strasburger established a strong presumption that the view of Krabbe was, after all, the right one. A re-investigation, however, seemed to us desirable, especially as the observations previously made by one of us had seemed to favour the opposite conclusion. We attached

[1] l. c. p. 339. [2] l. c. p. 333. [3] l. c. p. 334-338.

[4] Bd. 47, 1889, p. 701.

[5] Strasburger, Üb. den Bau u. die Verrichtungen der Leitungsbahnen in den Pflanzen; Histologische Beiträge, III, 1891, p. 400.

special importance to the direct examination of the stages of development as seen in longitudinal sections, in which alone the nascent tracheae can be observed in relation to the other elements of the bundle and of the ground-tissue. We found that the use of the microtome was of essential service. We obtained the best results by making continuous series of tangential sections through the region of secondary growth. In this way we were enabled to examine all the elements in each developing bundle, and to compare the various bundles at all stages of differentiation. The instrument chiefly used was the Cambridge rocking-microtome. The thickness of the sections in some series was $\frac{1}{5000}$ in. (·005 mm.), in others $\frac{1}{3333}$ in. (·0075 mm.). The usual paraffin-method was employed, and various stains were used, saffranin being on the whole the most successful. The result was of course checked by the constant comparison of transverse and radial sections, and also by means of maceration. Our work extended to several species both of *Yucca* and *Dracaena*, but our best results were obtained in an unnamed species of *Yucca*, in which we had abundant material of an old stem, reaching at least 3 in. in diameter. This stem had been in very active growth when preserved, and possessed a wide zone of thickening, with secondary bundles in all stages.

In this species the secondary bundles have the same structure as in the *Yucca* described by Röseler[1]; they are collateral, with the small phloëm-group on the outer side, lying in a depression of the much larger xylem-mass. The latter consists mainly of tracheides, with lignified parenchymatous cells scattered among them, and lying between the tracheides and the phloëm. The whole bundle is surrounded by a sheath of flattened, partially lignified cells, easily distinguishable from the comparatively thin-walled ground-tissue. We found that the average number of elements in the transverse section of a secondary bundle, taking the mean of twelve countings, is—tracheides 36, xylem-parenchyma 13, phloëm-elements 17.

The elements of the sheath average about fifteen in number.

[1] l. c. p. 297.

If we include them in the xylem-parenchyma, as Röseler appears to have done, we obtain figures which are not very different from his [1]. We shall return to these numbers later on; it is sufficient now to point out that, leaving the sheath out of consideration, more than half the elements in the mature bundle as seen in transverse section, are tracheides, while in the xylem they form about three-quarters of the whole number. In a tangential section passing through the middle region of a bundle the average number of tracheides cut through is about six. In such a section not more than two parenchymatous cells are likely to be met with at any one level. The tracheides in this species attain an average length of 2·78 mm. (mean of twelve measurements). The mean length of a cell of the secondary desmogen is ·075 mm. If therefore the entire tracheide is formed by cell-fusion, a series of thirty-seven desmogen-cells must, on the average, fuse to form each tracheide. If, on the other hand, the tracheides are formed by longitudinal growth alone, then each desmogen-cell which forms a tracheide must, on the average, grow to thirty-seven times its original length. The whole of this elongation would be by 'sliding-growth,' as we are speaking of a region in which the stem as a whole has long ceased to grow in length. On the hypothesis of cell-fusion we should find, in the developing bundle, as many rows of fusing desmogen-cells as there are tracheides at maturity, i. e. about thirty-six rows in the entire strand, or about six in any tangential section passing near the middle of the bundle. If, however, the development is by sliding-growth, we should find only *one* cell, on the average, at each level, in the whole strand, elongating in order to become a tracheide. A thin tangential section could of course only occasionally pass through one of these cells at the commencement of its elongation.

Now let us consider what we actually find on observing a continuous series of tangential sections through the zone of secondary increase.

Proceeding from without inwards, we first come, immediately

[1] l. c. p. 298.

on passing the cambium, to the earliest condition of the desmogen-strands. We can trace the first longitudinal divisions of the mother-cells from which these strands are formed. As is well known, it is usually a single longitudinal row of mother-cells, derived from the cambium, from which the entire strand is developed[1], though sometimes two adjacent rows may contribute to its formation. As we advance inwards the young desmogen continues to show a beautifully clear and simple structure. The outlines of every daughter-cell are clear and sharp, and the limits of their mother-cells can still be traced. All the desmogen-cells at this stage are alike; they are prismatic in form, with wedge-shaped ends fitting closely together. Each cell has a single nucleus, and all the nuclei are similar to one another. As we now proceed to examine sections rather further inwards, we find that the somewhat more advanced strands show a differentiation in two respects. (1) We notice every here and there an exceptionally large nucleus, often much elongated, and sharply distinguished from the small nuclei of other desmogen-cells. (2) We find that the regularity of structure of the desmogen is now at some places interrupted. Most of the regular prismatic cells remain unaltered, but occasionally we find among them a cell with more pointed ends projecting beyond those of neighbouring cells. Sometimes the ends of these elongated cells cannot be traced at all as they dip out of the plane of section. *These exceptional elements are invariably those with the specially large nuclei.* At first they are very rare, but as we advance to older bundles they are found more and more frequently. But now they are hardly ever found complete in a single section. Their length, and somewhat curved course, renders this impossible, and it is only by the comparison of successive sections that we are able to build up the entire element. As we proceed to yet older bundles we find that the greater part of them is now formed of these long, irregularly curved cells. Ultimately we recognise the latter in a changed condition; their walls are thicker, bordered pits

[1] Cf. Röseler, l. c. p. 322.

appear, and the cell-contents gradually vanish. As long, however, as the living body of the element persists, it possesses the single large nucleus by which it was at first distinguished.

While the young tracheides are going through these changes the other elements of the bundle undergo at first little modification. In fact, the strand of desmogen remains, but becomes gradually enveloped (except at the extreme outer edge) and partly permeated by the hypha-like tracheides. The latter grow in diameter as well as in length, and so come eventually to form much the greater part of the mass of the bundle,—a part altogether out of proportion to their number.

The developing tracheides have at first a denser protoplasm than the neighbouring cells, a fact which often helps greatly in recognizing them. In the later stages the denser protoplasm is found to be limited to their pointed ends, which we must suppose to be the seat of active growth.

In order to observe the stages of development satisfactorily it is essential to have very thin sections. This has the disadvantage that the complete tracheide is hardly ever contained in a single section. Hence the necessity for serial sections from which the form of the whole element can be reconstructed. It remains difficult to find suitable preparations for drawing. Fig. 1 is fairly satisfactory, but even here there is reason to believe that the extreme upper end of the tracheide is incomplete.

The developing tracheides often have a somewhat crooked course, as seen in tangential section. This would in itself agree well with their origin by cell-fusion, as the desmogen-cells of successive tiers do not lie in the same straight line, but roughly alternate with each other. It is evident, however, that the same course must also result from sliding-growth, for the growing tracheides must undergo a bend whenever they pass the points of junction of neighbouring cells, which are not in the same straight line. After the development of the tracheides has made some progress, the phloëm becomes differentiated at the outer side of each bundle. The sieve-

tubes become recognizable by their denser contents, and the perforation of the sieve-plates can be traced. It is important to recognize the phloëm at the earliest stage possible, as otherwise the young sieve-tubes might easily be taken for tracheides developing by cell-fusion, a mistake which we suspect has actually been made.

The careful study of serial tangential sections appears to us decisive against the hypothesis of cell-fusion, and in favour of that of sliding-growth. As regards the former point, the negative evidence is in itself very strong. More than half the bundle consists of tracheides. If they arose by cell-fusion therefore, this must take place on a great scale. In tangential sections through the middle of young bundles we should find most of the cells in various stages of fusion. Even supposing the fusion to take place so rapidly as to have been always missed by us (a very improbable supposition considering that the whole thickness of the growing zone was examined in many different series of sections), still at one stage we should find the xylem consisting of separate merismatic cells in rows, and at the next stage we should find it chiefly made up of continuous tubes, which must correspond exactly to the previous rows of cells. Either the nuclei must disappear or fuse, or they must be found in large numbers (thirty-seven on the average) in each tracheide. They certainly do not disappear till very late; for a long time we find a single large nucleus in each tracheide, which is even more conspicuous than those of the other cells. Nor will fusion explain this. The fusion of more than thirty nuclei in a vessel more than thirty times the length of its constituent cells could not possibly be a quick process, and its stages certainly could not escape observation. Nothing of the kind was ever seen.

The stages we actually find are: (1) the regular rows of desmogen-cells; (2) the same arrangement, but slightly disturbed here and there by the presence of a longer element among the shorter ones; (3) more disturbances of the primitive regularity, more elongated elements, the shorter cells more and more enveloped by them, until ultimately all the

inner part of the bundle is mainly formed of the long elements, with the short cells scattered among them.

So far the view that cell-fusion is the main factor in the development has been, as it seems to us, disproved; that a very large amount of sliding-growth must take part in the development of the tracheides, is certain. It remains to consider the possibility that a relatively short syncytium, i.e. the product of the fusion of a few cells, might grow out to form a tracheide. This view might seem to present fewer difficulties; the cell-fusions would be comparatively rare, and so much the easier to miss; there would be fewer nuclei to fuse in each element, and thus the apparent absence of fusion-stages might be explained away. So far as the evidence from transverse sections is concerned, Röseler admitted the possibility that the facts might be accounted for if about five cells fused to form each tracheide, and the resulting syncytium grew out to six or seven times its original length. If this happened in the species of *Yucca* we investigated there would be about six parallel rows of fusing cells in each young bundle.

This view is in reality as untenable as the hypothesis of entire development by cell-fusion. The single nucleus of the future tracheide is conspicuous throughout, until it is absorbed with the rest of the contents. If this nucleus arose by the union of several nuclei, some indications of this fusion must be found. Some of the growing tracheides observed were little longer than the desmogen-cells, so that there was no possibility of cell-fusion here. The idea of the outgrowing syncytium, though tempting in so far as it might seem to reconcile conflicting observations, must be rejected, from the entire absence of evidence in its support.

The development of the tracheides was investigated by the same method of serial sections in *Dracaena fragrans*, Gawl. and *D. angustifolia*, Roxb. In these the secondary bundles are concentric, the xylem completely surrounding the phloëm. The results entirely agree with those obtained in the *Yucca*. The material however was less favourable, as the zone of developing secondary tissues was narrower, and consequently

fewer stages of development were met with in each series. But so many young tracheides were observed at various stages of elongation, and always with a single nucleus, that there could be no doubt of their development by growth alone.

The results obtained from sections were confirmed by the method of maceration. The ordinary Schulze's mixture (nitric acid and potassium chlorate) was used. Thick tangential sections through the zone of secondary growth were treated with the reagent, and it was then found possible, in many cases, to isolate the elements of the developing bundle. In Fig. 2 a young tracheide of *Yucca sp.* is shown, which had only reached about a quarter of its mature length (·725 mm.). The single large nucleus is conspicuous. Some desmogen-cells of the same strand are shown for comparison. Fig. 3 represents a young macerated tracheide of *Dracaena fragrans.* Here the average length of the mature tracheide is 3·15 mm.; that of the young element shown is ·95 mm. It is obviously uninucleate. Here again desmogen-cells are shown for comparison. These results require no long description, as they simply repeat those obtained by Röseler.

Krabbe, Kny, Mdlle Lovèn, and Röseler, all lay great stress on the results obtained by counting the elements of the bundle, as seen in transverse section, at various stages. If the tracheides were formed entirely by cell-fusion it is obvious that the number of elements in the transverse section of the desmogen-strand, after the completion of its divisions, would be the same as in the mature bundle. If however the tracheides arise by elongation of single cells, the desmogen-strand must contain less than half as many elements as the mature bundle, in transverse section. Thus in our species of *Yucca* the average number of elements seen in mature bundles is sixty-six (neglecting the sheath). Of these, thirty-six are tracheides. The strand of desmogen when its divisions are completed must therefore show an average number of sixty-six elements on Kny's view. As, however, on the opposite supposition, only one cell at each level grows out to form a

tracheide, the desmogen-strand, after completed division, but before elongation of the tracheides, should show in transverse section an average number of thirty-one elements only.

Now this method seems at the first glance very promising; as a matter of fact it has led, in the hands of different observers, to absolutely contradictory results. Röseler, the last author who employed it, obtained, as we have seen, results seeming to point to a considerable amount of cell-fusion [1], a conclusion which is quite inconsistent with direct observations on serial sections or on macerated material. He found, in fact, too many elements in his supposed unaltered desmogen-strands; in the light of other observations we can easily explain this by supposing that a certain amount of sliding-growth of the tracheides had already taken place. This, however, is assuming the point to be proved, and the inefficiency of the counting-method by itself is manifest. Apart from the obvious difficulty arising from the great variability of the number of elements in different bundles (ranging from forty-four to ninety-four in the examples counted by us), there is the further difficulty that sliding-growth certainly begins before all the divisions have taken place, so that we have no fixed point at which to begin our numeration.

Although the counting method does not help us in detail, yet the comparison of transverse sections of developing bundles is very instructive. We find at a certain stage a small strand, with active cell-division in all parts. At a later stage the strand is larger, the external portion (in *Yucca*) remains unchanged, or may show some additional divisions. The more internal part, although the seat of the chief growth, shows no fresh cell-division whatever. Its increase is due to the intruding ends of young tracheides, growing in from other levels. Tracing the changes in older bundles we continue to find an enormous increase in the number of elements of the xylem of the bundle, as seen in transverse section, without any cell-divisions to account for it. This is only to be explained by sliding-growth, and in fact affords by itself, a

[1] Röseler, l. c. p. 333.

decisive proof of its occurrence as the chief factor in the development of the bundle.

Our final conclusions then as to the development of these tracheides are as follows:—

(1) The tracheides are formed by longitudinal growth only, each tracheide arising from a single cell, which may grow to 30—40 times its original length, but remains uninucleate throughout its whole development.

(2) As the secondary tracheides are formed in a region which has ceased to grow in length, their development is entirely by *sliding-growth*, and consequently the number of initial desmogen-cells from which they arise is very small. In the *Yucca* investigated, for example, only a single cell in each tier of the desmogen-strand can become a tracheide.

There can be no doubt that the development of the tracheides in the primary bundles is similar, but as the latter are formed in a region which is still lengthening as a whole, a proportionately smaller amount of sliding-growth is involved.

The process in the case of the secondary bundles is a highly remarkable one and vividly recalls the invasion of a tissue by the hyphae of a luxuriant parasitic fungus. The initial cells from which the tracheides develope, might be compared to the spores of the fungus. We tried in vain to determine the position in the desmogen-strand of the initial cells for the tracheides. They certainly do not form a continuous longitudinal row. We believe that they may occur in any part of the strand, with the obvious exception of that group which is destined to form the phloëm [1].

As our investigation has completely confirmed the views of Krabbe and Röseler, and the later view of Strasburger, it may be thought our duty to offer some explanation of the contradictory result obtained by Kny and Mdlle Lovèn, a result which one of us formerly accepted. From our own experience we think the chief sources of error are the following: The course of the tracheide as it passes between the overlapping and bevelled ends of the other elements of

[1] Cf. Krabbe, l. c. p. 64.

the strand often gives it just the same shape as if it had arisen by cell-fusion. When it bends aside to pass from one tier of cells to the next it is often constricted, and an oblique wall lying over it at such a point may easily simulate a perforated septum. Confusion of the very young sieve-tubes with developing tracheides is also possible, and would of course suggest cell-fusion. Accidental tearing of the delicate end-walls of the desmogen-cells may also give rise to deceptive appearances. The idea that the tracheides are multinucleate may have arisen partly from the confusion of coincident elements in different planes, partly from the presence in the tracheides of coagulated masses of protoplasm, which might be easily mistaken for nuclei. We know from our own experience that all these cases are possible sources of error; but we cannot undertake to explain how other observers may have been misled. In Kny's Fig. 2, Pl. XIV,[1] the long element to the right may probably be a developing tracheide, though not recognised as such by the author.

Appearances suggestive of cell-fusion are rare, and are most frequent in the least satisfactory preparations. We are convinced that such appearances are in all cases illusory.

We hope that the question may now be regarded as definitely settled, and that one of the most striking cases of sliding-growth in the development of vegetable tissues has thus been firmly established.

II. Secondary Growth in thickness of the Roots of Dracaena.

Until the year 1884 our knowledge of the development of secondary tissues in the roots of Monocotyledons was very meagre. The earliest account known to us is that given by Caspary in 1858[2]. He examined several species of *Dracaena*, and states that the cambial layer of the root lies between the 'Schutzscheide' (endodermis) and the central mass of vascular bundles; in modern terminology the cambium observed by

[1] l. c., Berichte d. deutsch. bot. Gesellsch. IV.

[2] Die Hydrilleen; Pringsheim's Jahrbüch., Bd. I p. 446.

him was pericyclic. He describes the bursting of the endodermis in consequence of the secondary growth. The short account given by De Bary is practically identical with this [1].

In 1884 Strasburger published an investigation of the roots of *Dracaena reflexa*, and showed that the cambium is first formed in the pericycle, and continues for a time to produce secondary tissues inside the endodermis, which thus becomes ruptured, but that sooner or later this activity ceases, while the cortical cells immediately outside the endodermis take up the division, and carry it on indefinitely [2]. The structure of the secondary tissues in the root is identical with that in the stem.

In 1885 Morot published his important paper on the pericycle. He examined the secondary thickening in the roots of various Dracaenas. He found that it usually takes place in the pericycle, but may exceptionally arise in the cortex. This may happen when there has been very little activity in the pericycle, and while the endodermis is still continuous [3].

In his book on the vascular tissues Strasburger confirms his former statements, and adds several interesting details. He finds that the xylem of the first-formed secondary bundles abuts in each case on two xylem-groups of the primary cylinder, thus enclosing one of the primary phloëm-groups. The strand of secondary phloëm is connected at its lower end (i. e. towards the root-apex) with a primary group. At the places where the endodermis is ruptured the internal and external tissues become perfectly continuous, and secondary bundles extend from the pericyclic into the cortical zone. The roots are epinastic as regards their secondary thickening, which begins on the upper side, and continues to be more vigorous there. The cambium is pericyclic near the

[1] Comparative Anatomy, English edition, p. 622.

[2] Das Bot. Practicum, 1st edition, 1884, p. 202. This account is omitted from the second edition.

[3] Morot, Recherches sur le péricycle, Ann. des Sci. Nat., Bot. Sér. 6. t. xx. p. 247.

apex of the root, and cortical in its older portion. These statements apply especially to *D. reflexa*[1].

Our own observations were made on the roots of *Dracaena fragrans*, Gawl., *D. Draco*, L., and *D. angustifolia*, Roxb. The last-named species was first examined, and in those roots which showed secondary thickening at all, we were surprised to find that it took place, from the first, entirely outside the endodermis, starting with the division of the first or second cortical layer. The pericycle was thick-walled, and neither had undergone any divisions, nor could do so at a later stage. So far as we saw there was no connection between the cylinder and the external vascular tissues. Our material, however, was limited, and it is probable that if we had been able to trace the tissues for some distance longitudinally, continuity through the endodermis would have been established.

These observations, however, showed that the accounts in the literature are not complete. It was evident that the cortical thickening might start on its own account, without any previous pericyclic divisions, at any rate in the same region of the root. Hence we were led to investigate the relation between pericyclic and cortical thickening more thoroughly than had previously been done.

Our best material was of *D. fragrans*, Gawl., of which we had a number of adventitious roots, up to almost an inch (2·3 cm.) in thickness, and showing secondary growth in all stages. Our most complete observations then were on this species.

As a rule, the cambium only appears in large adventitious roots, and in them only at a late stage. In a fully formed adventitious root about 1 cm. thick we may expect to find secondary growth beginning. This, however, is very variable, and in one case we found a complete zone of secondary tissue in a root only 4 mm. thick. The primary cylinder of the roots varies very much in structure. In the small root just mentioned, for example, it showed an ordinary polyarch

[1] Strasburger, Histologische Beiträge, III, 1891, pp. 403 and 508.

arrangement, such as is general in Monocotyledons, with a normal pith. In all the larger roots, however, the primary structure is much more complex, the pith being traversed by a variable number of xylem- and phloëm-strands, generally associated together, and imbedded in groups of sclerenchyma. These peculiarities have been described so often that we need not dwell on them here. Strasburger has shown that the medullary strands of the root are directly continuous with vascular bundles of the stem [1]. Transverse sections through roots in which secondary growth has begun may show three different conditions:—

(1) The cambium may have appeared in the pericycle, and the entire zone of thickening may be limited to the inside of the endodermis. This may still be the case in quite advanced stages, where there is a mass of secondary tissue, containing several concentric rows of bundles.

(2) There may have been no pericyclic divisions at all. In these cases the primary structure of the cylinder is unaltered; the secondary zone has been entirely superadded by the activity of a purely cortical cambium (see Fig. 5).

(3) We may find a mixed condition, the secondary growth having begun in the pericycle, and then having been taken up by a cambium formed in the cortex. This is the case described and figured by Strasburger [2]. The two processes are mixed up in the most irregular way. In one radial row of cells the pericyclic divisions will have gone on for a long time before the cortical activity supervenes; in the next row the pericyclic cambium ceases to act almost immediately, and nearly the whole growth is cortical. Hence we find fragments of the endodermis carried out into the secondary zone to a most variable distance (see Fig. 6).

The same transverse section may show only one condition, or may include two, or all three, in different parts of the periphery of the cylinder. Thus in Fig. 4 conditions (2) and (3) are shown; in Fig. 7, conditions (1) and (2), between which the

[1] Hist. Beiträge, III. p. 403.
[2] l. c., III, p. 404, Pl. V. Fig. 45.

transition happens to be a sharp one. In the mixed case (3) it is remarkable how thoroughly homogeneous the secondary tissues are, whether they are of pericyclic or cortical origin. The same bundle may pass out from the one zone into the other, one part being formed by the cortical, and the other by the pericyclic cambium. Some of the endodermal cells are still thin-walled when the secondary growth begins, and consequently are not easily recognisable when the displacements due to thickening have taken place. Hence the endodermis may appear to be ruptured at more points than is really the case. It is very probable that the thin-walled endodermal cells may themselves take part in the cambial divisions, as was noticed by Morot[1]. We did not find a clear case of this however. At the base of the adventitious root, near its insertion in the stem, it appears that the whole endodermis is thin-walled, and in advanced stages it is here impossible to make out the limit between pericyclic and cortical formations.

This peculiar mode of growth is really only a special case of the type of secondary thickening prevailing in Monocotyledons. There is not as a rule a single initial layer here, as there is in typical Dicotyledons and Gymnosperms[2]. The same cambial cell only continues active for a limited time, and then the divisions are taken up by an adjacent cell towards the exterior[3]. An extreme illustration of this process is afforded by Fig. 8, which shows an early stage of purely cortical growth in thickness in the root of *D. Draco*. Here three or even four distinct rows of cortical cells have already taken up the cell-division. It is essentially the same phenomenon when pericyclic is succeeded by cortical divisions, only here there is usually a thick-walled endodermis to be overleapt. If this physiological barrier were really continuous it would probably be an effectual obstacle to any such mode of growth. We know, however, that it is not absolutely

[1] l. c. p. 248.

[2] Some doubt has been recently cast on the constancy of the initial layer even in these classes; see Raatz, Stabbildungen im secundären Holzkörper und die Initialentheorie, Pringsheim's Jahrbüch., XXIII. 1892.

[3] Cf. Strasburger, Hist. Beiträge, III, p. 396. Röseler, l. c. p. 309.

continuous, though it may be so for long distances. That the divisions should pay no respect to the morphological distinction between stele and cortex cannot surprise us[1].

The case where the cambium at once appears outside the endodermis is more puzzling. Here both pericycle and endodermis may be very thick-walled, and, so far as the transverse section shows, there may be no interruption to their continuity. The secondary tissues therefore are from the first cut off from any direct communication with the primary cylinder at the same level. In this case it is by no means always the *first* cortical layer which divides; sometimes it is even the fourth layer from the endodermis in which the first divisions appear.

It is impossible to understand this type of secondary growth unless we trace the course of the cambium and its products in the longitudinal direction. The statements in the literature appear to show that the secondary tissues taper off regularly towards the root-apex, their maximum thickness being at the base of the root, where the process presumably started in the first instance. It has also been noticed that the thickening is almost always highly excentric, the upper side of the approximately horizontal root, according to Strasburger, receiving the first and greatest increment. The thickening near the apex is said to be always pericyclic, cortical cambium first appearing in a more advanced region.

Although this account of the process is no doubt applicable to certain cases, or it may be to certain species, we have not found it confirmed by our observations on *Dracaena fragrans* and *D. Draco*.

We only found one instance in which the secondary growth appeared to have started from the base only of the adventitious root and advanced regularly towards its apex. This was in *D. fragrans*, and in this case the thickening remained pericyclic throughout. In all other thickened roots of these two species which we investigated (and in *D. fragrans* they were fairly numerous) the maximum thickness of the secondary

[1] Cf. Scott and Brebner, On Internal Phloëm, Annals of Botany, vol. v. p. 287.

zone was attained, not at the base of the main adventitious root itself, but at the insertion upon it of a branch-root. From this point the secondary tissues thinned out both in the upward and downward direction, and also peripherally. Near the insertion of the lateral root we constantly found pericyclic thickening; at a distance from it, in whatever direction, the secondary zone was invariably formed in the cortex. The transition was in some cases a sudden one; more usually it was gradual, and in the intermediate region both pericyclic and cortical thickening had taken place.

Further, we found that in these cases the thickness of the secondary zone bore no relation to the upper or lower side of the root, but was always greatest near the insertion of a branch root, wherever the latter might arise.

We will describe a particular case more in detail. In a root of *D. fragrans*, 2·3 cm. in diameter, we examined radial sections passing through the base of a branch-root. At the insertion of the latter there had been abundant secondary development. There are numerous zones of secondary bundles, the inner of which slope obliquely outwards, forming a connection with the tissues of the branch-root. The outer secondary bundles have an approximately longitudinal course. A normal zone of thickening has in fact been built up upon the secondary network of bundles belonging to the base of the branch. The whole of this secondary mass has been formed by a pericyclic cambium; the endodermis lies entirely outside the secondary tissues. As we recede from the insertion of the rootlet in either direction, the thickness of the secondary zone gradually diminishes. The endodermis curves rapidly inwards, passing obliquely through the secondary tissues, which in this region have been formed partly within it and partly to the outside. Further on still the endodermis takes a straight course, and borders directly on the primary cylinder. Here the thickening has been exclusively cortical. In the transitional region the endodermis is often interrupted, and sometimes we could trace a secondary bundle through it from the inner into the outer zone. As regards the actual dimen-

sions, in one case measured, the thickening had become purely cortical at a distance of 1·3 cm. from the nearest part of the base of the branch-root. The length of the region of transition renders it inconvenient to figure on an adequate scale; consequently we have contented ourselves with reproducing a corresponding transverse section (Fig. 7), to which we shall return.

If a transverse section be taken at some distance from a lateral root (say 1–1·5 cm. above or below), only cortical secondary tissues are shown. They may extend all round the cylinder or be limited to the side on which the lateral root is situated; that depends on the amount of progress which the thickening has made. In any case, however, the zone is thicker on the side corresponding to the branch-root. If the transverse section be made nearer the latter, we find both pericyclic and cortical secondary growth at one side, which as it thins out to the right and left becomes purely cortical (see Fig. 4). Lastly, if the section pass through, or close to the insertion of the lateral root, we find pericyclic thickening next the insertion, and cortical at a distance from it, with a more or less gradual transition between them. There are many variations in detail, of which Fig. 4 and Fig. 7 will give some idea; Fig. 5 is from a region of purely cortical thickening, at a distance from a lateral root, while Fig. 6 is from the transitional region. In Fig. 7 the transition is unusually sudden, causing a sharp break in the endodermis, reminding one of a geological 'fault.'

If our sections be cut from comparatively young roots, we find only the pericyclic thickening near the base of the lateral roots. It thins out altogether at a distance from them, and the cortical thickening has not yet begun.

It may be worth while to mention that in one large root, 2·1 cm. in diameter, where the secondary tissues extended round the whole circumference, their maximum thickness, on the side towards the lateral root, was 3·5 mm., the minimum thickness, on the side opposite the lateral root, was ·5 mm. In this case there was some pericyclic thickening all the way

round, but on the side remote from the branch-root it became very small in amount, and almost wholly parenchymatous. Nearly the whole of the secondary zone in this part was of cortical origin.

So far we have established a definite relation between the secondary thickening and the insertions of the branch-roots. The observations which we made on *D. Draco* confirmed those on *D. fragrans*. In one root of the former we found, on examining radial sections, that no sooner had the secondary tissues begun to thin out in receding from a lateral root, than they began to widen again as the next lateral root was approached. In this case the whole thickening was pericyclic, the cortical stage of growth never being reached between the two rootlets.

A strong presumption has been established that the secondary increase actually starts from the insertion of the rootlets. In fact, the younger stages in which we have pericyclic thickening only, limited to the immediate neighbourhood of the rootlet, raise this presumption almost to a certainty. The usual case appears to be that the cambium forms in the pericycle at the insertion of the rootlet, and that the divisions spread gradually in all directions, but at first are limited to the layer in which they started. As secondary tissue is formed the continuity of the endodermis is broken, and at the place where it is interrupted the divisions are taken up by the neighbouring cortical cells. By the time that the cambial activity has extended to the more remote parts of the root, the pericycle in those parts will have usually become thick-walled and incapable of division. Hence at a certain distance from the rootlet only cortical cambium can as a rule arise. In one case we were so fortunate as to observe, in radial section, the very first cambial divisions in the cortex. They took place immediately outside the place where the endodermis was ruptured, near the base of a rootlet. The dividing cells were thus in direct communication with the pericyclic cambium. When once started, the cortical divisions can spread up or down the

root independently of any further communication with the pericycle.

It is not probable that *all* lateral roots form the starting-points for secondary growth. In some cases they showed no secondary bundles at the base (though there was some radially arranged parenchyma) and no signs of cambium.

The connection between the internal and external tissues is established partly on a large scale, where the two zones are continuous, through large gaps in the endodermis of the transitional region; partly on a small scale by local connections We have several times seen a single secondary bundle passing through the endodermis, and in one case we found a horizontal strand of tracheides connecting the primary xylem of the cylinder with the secondary tissues which had been formed in the cortex. We can confirm Strasburger's statement that vessels only occur in the *primary* xylem-groups of the roots. The secondary bundles are quite like those of the stem, and their water-conducting elements are invariably tracheides.

It appears, then, that in most of the roots investigated by us the formation of secondary tissues starts from the insertion of the rootlets, and at first serves to establish additional channels of conduction between the branch and its parent organ. Subsequently the process of new formation thus initiated extends to all parts of the root. An acropetal formation of secondary tissues, starting from the base of the adventitious root itself, also occurs, but does not extend far, and serves to establish the connecting link between the scondary tissues of the root and those of the stem.

It may be mentioned that the cambium in the root often forms several layers of secondary cortex on its outer face.

Periderm is regularly formed in these roots, sometimes from the layer next within the exodermis, sometimes from a deeper layer of the cortex.

Our results may be summed up as follows:—

(1) In the adventitious roots of *Dracaena fragrans* and *D. Draco*, the secondary growth in thickness starts from

a number of distinct points. One of these starting-points may be the base of the root itself. The chief formation of secondary tissues, however, begins at the bases of rootlets, and thence extends both up and down the root, and also peripherally.

(2) At the base of the rootlet the thickening takes place entirely by means of a pericyclic cambium. At a distance from it there is usually only cortical cambium, and consequently the whole of the secondary tissues are here external to the endodermis. In the transitional region there may be first a pericyclic, then a cortical cambium, and the secondary tissues are here formed on both sides of the endodermis.

(3) The connection between the vascular tissues inside and outside the endodermis is not only maintained through the transitional region, but also by means of special bundles which traverse the endodermis at various points.

(4) The important part played by the cortex in the formation of secondary vascular tissues in these roots, shows that the morphological distinction between central cylinder and cortex is not necessarily correlated with a permanent difference of function.

III. The Secondary Growth in Thickness of Aristea Corymbosa, Benth. (N.O. Irideae).

Within the natural order Irideae, which now includes between 900 and 1000 known species, there is a little group of shrubby forms. Only four such species are at present known to science; all belong to the tribe Sisyrinchieae, subtribe Aristeae, and all are natives of the south-western provinces of the Cape Colony. The plants in question are *Aristea fruticosa*, Pers., *A. corymbosa*, Benth., *Witsenia maura*, Thunb., and *Klattia partita*, Baker. The two first-named species now form the subgenus *Nivenia* of the genus *Aristea*, of which twenty-seven species are known in all. *Witsenia*, Thunb., as at present limited, and *Klattia*, Baker, are both monotypic genera[1].

[1] See Baker, Handbook of the Irideae, 1892, pp. 145 and 146, where the

We are not aware that the anatomy of the stem in any of these species has so far been described. Professor F. O. Bower, F.R.S., first called our attention to the occurrence of secondary growth in thickness in *Aristea corymbosa*, and to him we are also indebted for the supply of abundant fresh material from the Glasgow Botanic Garden.

Aristea corymbosa is a low shrub; the stems are elongated, much branched, and cylindrical; the younger branches are flattened in the plane of the distichous leaves, which are equitant, linear, rigid, and erecto-patent, attaining a length of from 4 to 6 inches[1].

It may be mentioned at once that the external characters of the other three shrubby Irideae are very similar to that of our species. *Aristea fruticosa*, Pers., is a dwarf under-shrub, much smaller in all its parts than *A. corymbosa*. *Witsenia maura*, Thunb., on the other hand, is a tall plant, with woody erect stems 2–4 feet long; *Klattia partita*, Baker, is perhaps the most like *Aristea corymbosa* in appearance; its woody, branched stems are 1–2 feet in length. All four species agree in their distichous equitant leaves, and flattened branches which become cylindrical with advancing age. It is highly probable that the account of the anatomical structure and development which we are about to give in the case of *A. corymbosa* will be found to hold good in essentials for all these shrubby forms.

1. *Primary Structure.*—We will begin with a short description of the primary structure.

The equitant leaves are in their upper ensiform portion typically centric in structure, with assimilating tissue and stomata on both sides. The collateral vascular bundles form a flattened ring, the xylem in each facing towards the interior,

synonyms will be found; also Baker, Systema Iridacearum, Linn. Soc. Journal, Bot., vol. xvi. 1878, pp. 108–110; Bentham and Hooker, Genera Plantarum, vol. iii. 1883, pp. 701, 702.

[1] The above is a slight extension of Baker's description, Handbook of Irideae, p. 145.

the phloëm towards the nearest surface of the leaf. Occasionally concentric (amphivasal) bundles are found in the middle of the mesophyll. The bundles forming the ring each have a stout strand of sclerenchyma outside the phloëm. Isolated strands or plates of sclerenchyma are also present in the leaf, especially at its edges. The central part of the mesophyll is colourless.

The leaf-base, which completely embraces the stem, has of course a different structure, and is in fact bifacial. We only found stomata on the outer (morphologically lower) surface, to which also the assimilating tissue is limited. The xylem of the bundles is here directed towards the upper surface. The sclerenchyma is mainly towards the upper surface, where it forms a continuous hypodermal layer.

The leaf-base has about twenty bundles altogether. The two largest are both median, lying one behind the other in the same radial plane. The other bundles are mostly of fairly uniform size, but become gradually smaller in the posterior direction. There are a few much smaller scattered bundles, usually placed further to the exterior than the main ones.

The leaf-traces on entering the stem curve in towards the middle of the cylinder, and then very gradually pass outward again, fusion between the bundles taking place towards their lower ends. In other words, the course of the bundles belongs to the familiar Palm-type. The larger bundles penetrate most deeply into the cylinder. The upper median bundle on entering the stem turns sharply upwards, and then as sharply down again, to take the usual course into the cylinder. We found that the number of vascular bundles in the transverse section of the primary cylinder of the stem averages about seven times the number of bundles in a leaf-base. Hence we may infer that on the average the bundles pursue a separate course through about seven internodes. It is probable, however, that this varies greatly, even among the bundles from the same leaf.

If we examine a transverse section of the flat stem, in a region where living leaves are still present, we find the

following structure (see Fig. 9). The middle part of the stem is occupied by a well-marked central cylinder, of lenticular section, which presents the ordinary characters of monocotyledonous structure. The scattered vascular bundles (which number 140 or more) are of extremely unequal size. The ground-tissue of the cylinder is thin-walled in its inner part, but becomes sclerotic towards the exterior. The cortex is conspicuously thicker at the ends than at the sides of the section, so that the stem as a whole is more strongly flattened than is the stele. The cortex is traversed by leaf-trace bundles. They are collateral here, as they are in the leaf, the xylem only partly embracing the phloëm. As soon as the bundle enters the cylinder, however, it becomes concentric.

If we now consider the structure of the primary cylinder rather more in detail, the first point to be noticed is that the vascular bundles differ among themselves in structure as well as in dimensions. Only a few of the larger bundles have any definite group of protoxylem. Of all the bundles in the cylinder perhaps one-eighth possess protoxylem (see Fig. 12, *px*). When present it occupies the usual position on the proximal[1] side of the strand. The large and small bundles are scattered irregularly throughout the cylinder; the larger, however, are more frequent towards the middle, the smaller towards the outside, to which part the smallest of all are limited. Several large bundles are always grouped near the centre, sometimes forming a ring around a central point of the ground-tissue which might be called pith. Of these inner bundles some, but not all, have protoxylem.

The bundles with protoxylem are those which are differentiated earliest, namely the upper parts of the principal traces. The lower portions of the latter and the finer

[1] The terms *inner* and *outer* are confusing in this connection as it is often doubtful whether they refer to the individual vascular bundle, or to the stem as a whole. We therefore propose to use the word *proximal* for that side of the bundle (or other structure) which is turned towards the centre of the axis, *distal* for that side which is remote from the centre.

bundles in their whole length are differentiated later, and have no protoxylem-elements[1].

The protoxylem has the usual spiral structure; we did not determine whether its elements are vessels or tracheides. The later-formed xylem (which is alone present in most of the bundles) contains tracheides only, with recticulated or pitted walls. A certain amount of xylem-parenchyma is present among the tracheides. The phloëm calls for no special description; the elements bordering on the xylem are parenchymatous; the central group consists of sieve-tubes and companion-cells.

The ground-tissue of the cylinder consists of rather elongated parenchymatous cells. Those surrounding the bundles are thicker walled and often prosenchymatous. Towards the outside of the cylinder the whole ground-tissue assumes the latter character.

The cortex is thin-walled throughout; many of its cells contain tannin.

2. *Secondary Tissues.*—If we next examine a transverse section through an older part of the stem, which has already assumed a cylindrical form, we find a very different structure (see Fig. 10). In the middle part of the section we recognize the lenticular outline of the primary cylinder, which is unaltered. But superadded on this we find an entirely new zone of tissue. Its maximum radius is at right angles to the major axis of the primary cylinder. Hence the effect of the addition of the secondary zone has been to give a circular section to the entire vascular system. At the same time the transverse section of the stem as a whole also becomes circular; this is assisted by the formation of periderm, which has been produced near the surface of the flat sides of the stem, but in a more internal position opposite its prominent edges. At the stage figured in Fig. 10 the cortex outside the periderm still remains; later on it is thrown off altogether.

[1] Cf. Strasburger, Hist. Beiträge, III, p. 398; also Röseler, l. c. p. 295.

The secondary tissues form two distinct regions of conspicuously different structure. The outer zone is characterized by scattered, sharply defined secondary bundles imbedded in comparatively thin-walled, radially arranged parenchyma. The inner secondary zone has the bundles densely crowded, so as not to be readily distinguishable, with but little parenchyma between them (see Fig. 10). Here there is no obvious radial arrangement. We will first describe in detail the structure of the outer zone.

On its exterior side it is surrounded by a regular cambial layer which is manifestly the seat of formation of the secondary tissues (see Fig. 14). The details of development will be considered later. On its outside the cambium produces secondary cortex, which eventually grows to a great thickness (see longitudinal section, Fig. 15).

The secondary vascular bundles, like all other bundles in the stele, are concentric. The ring of xylem consists chiefly of long tracheides, with a very tortuous course. Their walls have corresponding bordered pits with slit-like openings; among the tracheides a few parenchymatous elements are scattered, some of which border on the phloëm. The latter presents no peculiarities; as the constituent elements of the sieve-tubes are short, their sieve-plates, which are horizontal, are often met with in transverse sections. It is very common to find two groups of phloëm in the same bundle; they may be placed either tangentially or radially. This is due to the fact that the secondary bundles often anastomose in both planes, as is easily seen in the corresponding longitudinal sections. The system of secondary bundles thus forms a continuous network.

The tracheides form much the greater part of the bundle. We found the average numbers to be for each bundle, as seen in transverse section, forty tracheides, nine cells of the xylem-parenchyma, and eight phloëm-elements. The rectangular pitted cells of the secondary ground-tissue have a very regular radial arrangement, which is only disturbed where the vascular bundles occur. The latter are arranged generally in concentric series.

It will be seen that this outer secondary zone shows a general agreement with the corresponding tissues of a *Dracaena*.

The inner zone has a more remarkable structure. The phloëm-groups stand out plainly enough, but the outer limits of the bundles are often impossible to trace. The whole appearance rather suggests some anomalous Dicotyledon with 'phloëm-islands' imbedded in a continuous mass of wood. The bundles are in fact to a great extent confluent, and are only here and there separated by a radial or tangential row of parenchymatous cells. The great bulk of the tissue in this zone consists of the tracheides of the crowded bundles. Consequently it is not surprising to find that no regular radial arrangement is evident. This is also partly accounted for by the mode of development, which will be explained below. The whole structure is the expression of a network of bundles, with thick strands and nodes, and very small meshes (occupied by parenchyma) between them.

The limit between the outer and inner secondary zones is a fairly sharp one. So also is the boundary of the primary cylinder, which is easily distinguished by its scattered bundles, circular in transverse section, imbedded in sclerotic ground-tissue.

This will perhaps be the best place to say a few words as to the periderm and the secondary cortex.

The first periderm forms at about the time when secondary thickening begins. The seat of its formation at the sides of the flattened stem is the hypodermal layer. Towards the narrow edges of the stem the next inner layer of the cortex takes up the division, then a more internal layer, and so on. Thus opposite the prominent edges of the stem the phellogen is deep-seated, and gives rise from the first to internal periderm. The thickness of the cortical layer within the periderm is consequently about the same all round the stem.

The phellogen usually forms two layers of phelloderm on

its inner side, and many layers of cork towards the exterior. The cells cut off by the phellogen often undergo further division by oblique walls, which may cause some disturbance of the normal radial arrangement.

As the stem grows older, successive internal periderms are formed, until the whole of the primary cortex is cut off. This does not happen however until the outer zone of thickening has made good progress. Thus at the stage shown in Fig. 14, some primary cortex is still left.

The formation of secondary cortex from the cambium does not begin until after the inner zone of wood[1] has been completed. During the development of the outer zone of wood, secondary cortex is formed with increasing rapidity (cf. Figs. 14 and 15; the latter was from the oldest part of the stem at our disposal). It may attain a thickness of about twenty layers before the primary cortex is lost. Ultimately the latter is cut off by periderm, and henceforth the entire cortex is secondary. The formation of internal periderms does not stop here however. The periderm shown in Fig. 15 has evidently been formed in the secondary cortex itself. As new cortex is formed from the cambium, the older layers are constantly removed by more internal periderms.

The periderm is provided with lenticels, but we did not follow their development. We may mention here that we found distinct indications of an abscission-layer at the base of the leaves. This subject also requires further investigation.

3. *Development of the Primary and Secondary Tissues.*— It is clearly of importance to determine whether the formation of the secondary tissues is a mere continuation of the primary development (Sanio's 'Thickening Ring' having an unlimited activity), or whether the cambium is an entirely secondary meristem, arising by the division of cells

[1] We use the term *wood* here for all the secondary tissue formed centrifugally on the inner side of the cambium. This is sanctioned by the authority of De Bary (Comparative Anatomy, Eng. ed. p. 591), but a better terminology is much needed.

which have already assumed the character of permanent tissue.

With this object in view we traced the development of the stem as shown in a number of transverse sections, made at measured distances from the apex. Several such series were examined, and the results checked by comparison with longitudinal sections. We will base our description in the first instance on a vigorous stem, in which we traced the differentiation of tissues from the apex downwards for a distance of 5·2 cm. Of course it will be understood that the absolute distances from the apex have no general value, and would come out very much smaller in less vigorous branches.

At about 1 mm. from the apex [1], nearly all the bundles of the cylinder are very oblique, the internodes not having lengthened much as yet. The leaf-trace bundles entering through the cortex are the most developed; the larger of them have their protophloëm and protoxylem already differentiated. The smaller leaf-trace bundles, however, even in the cortex, are still quite in the procambial [2] condition. In the cylinder too only the largest bundles (obviously belonging to the upper portions of the principal leaf-traces) show a differentiation of the first xylem- and phloëm-elements; all the rest are procambial. There is no regular centrifugal order in the development of the different bundles in the cylinder. As however the smaller leaf-traces, and the lower ends of the principal ones, are limited to the outer part of the cylinder, it is here that we find the largest proportion of procambial strands, some of the outermost of which are in the very earliest stages of formation. In the outer zone of the cylinder active cell-division is in progress, especially towards the edges of the flat stem, and new bundles are being originated.

[1] We did not concern ourselves with the actual growing-point, the investigation of which has no bearing on our main question.

[2] We use *procambium* and *primary desmogen* as synonymous terms. Our use of the term *secondary desmogen* has been already explained (p. 22).

The cortex and epidermis are already nearly fully formed, and in the former the tannin-sacs have acquired their characteristic contents. The stage shown in Fig. 11 is at about 2 mm. from the apex. At this distance the bundles of the cylinder have become more straightened, owing to the elongation of the internodes, and so better sections can be obtained. Otherwise there is not much change. The divisions at the periphery of the cylinder (Sanio's 'Thickening Ring') take place irregularly in all directions.

At 5 mm. from the apex considerable progress has been made. The primary development of the cylinder as a whole has almost ceased. Scarcely any fresh divisions are now found in the 'Thickening Ring.'

Many of the outermost bundles of the cylinder, however, are still in an early procambial stage. The principal bundles appear to have their phloëm fully formed. The proximal part of their xylem has been completed in the usual centrifugal order. A few of the elements of the distal half of the xylem-ring are also becoming lignified. In this part of the bundle the differentiation of the xylem-elements follows no definite order. We may speak of this later-formed, non-centrifugal part of the xylem as *metaxylem*[1]. In the large bundles the phloëm follows the normal centripetal order of differentiation. In all the other bundles (forming the great majority) the whole of the xylem is metaxylem. There are no spiral elements, and there is no centrifugal development. Differentiation begins indiscriminately at any points of the xylem-ring, and no preference whatever is shown for its proximal side. So too with the phloëm; in these later-developed bundles the phloëm does not develope centripetally; so far as any regular order can be traced, the phloëm-elements in the middle of the bundle appear to be completed first.

[1] The term was introduced by Van Tieghem for that part of the primary xylem in the root which is differentiated after the normal centripetal development is completed; see his Traité de Botanique, 2nd ed. p. 684. *Mutatis mutandis* the same term may well be applied to late-formed *non-centrifugal* xylem in a bundle belonging to the stem.

Fig. 12 is drawn from a section at 5 mm. from the apex. The differentiation of the bundles with metaxylem only, takes place rather later, and was studied in other series of sections.

Returning to our first stem, we find at 23 mm. from the apex that the primary development of tissues is completed, and we have the normal structure of the central cylinder in its fully differentiated condition. This is the stage at which development would cease, if we were dealing with an ordinary Monocotyledon. Two points, however, must be noticed. Near the periphery of the cylinder, bordering directly on the pericycle, we still find a few unfinished bundles; some are quite procambial, in others about the proximal half of both xylem and phloëm is differentiated. Secondly, in the pericycle itself we find here and there a very few scattered cells in which a recent tangential division has taken place. These two points indicate that a further process of development is still to follow. Otherwise the structure has been sufficiently described at p. 47. At 31 mm. from the apex we found the tangential divisions in the pericycle more frequent, and on one side of the cylinder the dividing cells formed a continuous tangential band, which already suggested a cambium.

At 41 mm. the cambium was well established in the pericycle, on both sides of the cylinder, but not at its ends (as seen in transverse section). At this stage we determined a fact which was confirmed by many other observations; the tangential divisions are not limited to a single layer of pericyclic cells, but two such layers may begin to divide simultaneously. Hence, from the very first, there is no single initial layer present. It will be remembered that a perfectly similar fact was noticed in the roots of *Dracaena* (cf. Fig. 8).

At 47 mm. from the apex we found that the development had reached a very instructive stage. The formation of secondary tissues had just been started. In one place a few tracheides had been added to complete one of the unfinished primary bundles; at another a new, altogether secondary bundle had been started. In fact, after the long pause, tissue-

formation has again fairly commenced, and the secondary period of growth is entered upon.

A stage similar to this (from another series) is shown in Fig. 13. At least two of the original pericyclic cells may contribute by their divisions to form one secondary bundle.

At the end of our series, at 52 mm. from the apex, the secondary zone is slightly more advanced.

The whole series is most instructive, showing that there is a long interval between the cessation of the primary development and the commencement of secondary increase. At 5 mm. from the apex the primary merismatic divisions had almost ceased; only at 31 mm. had anything approaching to a continuous cambium arisen by fresh divisions, and the first formation of secondary tissues did not begin until a distance of 47 mm. from the apex was reached. There is thus a perfectly definite distinction between the primary and secondary tissues, though individual vascular bundles may be common to both, as indeed is necessary in order to keep up physiological continuity.

In feeble branches the interval between primary and secondary development is much less marked, and may even be almost obliterated. This is evidently due to a 'telescoping' of the developmental stages, and does not affect the conclusions drawn from vigorous shoots, to which we must look for the typical mode of growth.

We will now complete, from another series, our account of the secondary development.

The mode of formation of the inner zone of thickening is peculiar. No regular radial series can be traced, and in fact there is no single continuously active layer of cambium. A cell of the pericycle divides up a few times—say six—by tangential walls formed in centrifugal order; the daughter-cells subdivide to form the elements of a bundle, or may directly become cells of the secondary ground-tissue. Meanwhile another pericyclic cell, on the distal side of the first, has begun to divide; this contributes its share, and then in turn its activity ceases, and so on. Hence the fully formed

elements of the secondary 'wood' do not necessarily fit on, in any way, to the cambial cells bordering on them externally. From the irregular character of this inner secondary zone, and the marked absence of any definite initial layer, one might be tempted to doubt whether this tissue can properly be called secondary, and whether it may not simply form the completion of the primary cylinder. The long interval, in normal cases, before the formation of the zone in question begins, an interval during which the primary cylinder has become fully differentiated, negatives any such idea, which is also inconsistent with the fact that the bundles of this zone are cauline, and only indirectly connected with the leaf-traces.

The development of the inner secondary zone appears to go on slowly. In a piece of stem 10 cm. long it was just commencing at the top, and just completed at the bottom. Hence the completion of this zone must have taken place at a distance of about 15 cm. from the apex. (Cf. p. 55.) It is possible that this may correspond to one year's growth, but of this we could obtain no evidence.

Not till the inner zone of thickening is nearly completed does the cambium extend round the ends of the cylinder (in transverse section); consequently there is never more than a thin layer of tissue belonging to this zone at these points. Its maximum thickness is at the middle of the broader sides of the stem (see Fig. 10).

When the transition to the outer secondary zone takes place the cambial divisions become more regular, and we find longer continuous radial series of cells. Henceforward the development goes on quite normally, and so far as we could tell the same cambium is active throughout. The normal process of secondary thickening is now established, and continues year after year. It is not, however, until after several series of the distinct secondary bundles have been formed, that any appreciable amount of secondary cortex begins to be developed.

As regards the details of development of the secondary

bundles of the outer zone, it is rare for the whole bundle to be formed from a single radial series. Usually two such series take part in its formation, sometimes one row forms the median part of the bundle, while the two adjacent rows cut off cells which contribute to form its flanks; sometimes all three rows contribute equally, but this perhaps only happens when an anastomosis is to be formed. The phloëm of each bundle is formed very early. A cell cut off on the inner side of the cambium divides up by two or three inclined longitudinal walls. Thus a little group of small cells is formed, which represents the future phloëm. Almost simultaneously an inner cell of the same radial row divides up to form the proximal part of the xylem. Next, cells situated at the sides, and either derived from the same radial series or from adjacent ones, take up the divisions, and form the lateral portions of the future ring of xylem. Lastly, fresh cells are added by the cambium to the distal side of the strand, and these ultimately complete the xylem towards the exterior. Of course all the stages run into one another, but it appears to be the rule for the phloëm to take the lead.

We had the same question to face here as in *Dracaena* and *Yucca*; how do the tracheides of the secondary bundles develope? The answer is here also a perfectly definite one; they develope by sliding-growth alone. The comparison of transverse sections is never conclusive by itself, but it affords valuable indications. We can say at any rate this much; not more than about twenty elements, as seen in transverse section, are, on the average, formed in the desmogen-strand by division. We know that the average number of elements in the transverse sections of a mature bundle is fifty-seven, of which forty are tracheides. The extraordinarily tortuous course of the tracheides, which form a sort of twisted skein in which the isolated cells of the xylem-parenchyma are entangled, and by which the phloëm is enveloped, also strongly suggests an origin by longitudinal growth in a confined space (see Fig. 15).

But direct evidence is not wanting. In longitudinal sections isolated desmogen-cells are sometimes found which have grown

to a much greater length than their neighbours. They remain uninucleate. Several stages were observed between the ordinary desmogen-cell and the fully formed tracheide. The course of the tracheides, however, renders it impossible to trace them far in sections. Hence recourse was had to maceration, and Fig. 16 represents a couple of very young tracheides, one isolated, the other still in connection with some desmogen-cells, which were obtained by this method. The process of development is undoubtedly the same here as in *Yucca* and *Dracaena*. In Fig. 17, the whole length of a mature tracheide is shown, in two halves. It can be compared with the desmogen-cells and young tracheides in Fig. 16, which are shown on double the scale of Fig. 17.

The sliding-growth begins very early, and has already made considerable progress in the proximal half of the xylem, while cell-divisions are still going on in its distal portion.

4. *The Roots.*—We found no secondary thickening in the adventitious roots at our disposal. From our experience in *Dracaena*, we paid special attention to the points of insertion of rootlets, but here also no signs of cambium were present. In fact, we may say for certain that the roots examined by us never could have formed a secondary zone. The whole of the cortex was already dying away, and the wide pericycle (8–10 layers in thickness) was too sclerotic ever to become the seat of a secondary meristem. The roots are of polyarch structure, and call for no detailed description. It may be mentioned, however, that they contain true vessels of large size, whereas vessels, with the possible exception of protoxylem-elements, are altogether absent from the other organs of the plant. The larger vessels of the root may be either reticulated or have bordered pits. They have inclined scalariform terminal walls; we demonstrated their perforation by injection with French Blue under pressure.

This occurrence of large vessels in the root only is also characteristic of *Dracaena*.

5. *Summary.*—Our chief results respecting *Aristea* are the following:—

(1) *Aristea corymbosa*, Benth., in common no doubt with the few other shrubby species of Irideae, forms an indefinite amount of secondary tissue by means of cambium, which continues active during the whole life of the plant.

(2) The tissues formed centrifugally, on the inner side of the cambium, consist of secondary concentric bundles, imbedded in ground-tissue; on the outer side of the cambium a large amount of secondary cortex is formed. The latter is wholly parenchymatous.

(3) The xylem of the secondary bundles consists chiefly of tracheides, each of which arises, as in *Yucca* and *Dracaena*, by the enormous elongation of a single cell.

(4) The cambium arises in the pericycle, and is a new formation; the cambial divisions do not begin until some time after the development of the primary vascular cylinder is completed.

(5) The inner zone of secondary tissues is characterized by its very crowded bundles. The cambium which forms this zone has no definite initial layer; each cambial cell undergoes a few centrifugal tangential divisions, then its activity ceases, and the divisions are taken up by an adjacent cell to the exterior. Consequently the elements of the inner zone do not show a regular radial arrangement.

(6) After a time (possibly, under normal circumstances in the second year) the divisions become more regular, a cambium with a definite initial layer is established, and the formation of the outer zone of thickening begins, and continues without limit. This zone is characterized by its scattered bundles imbedded in comparatively thin-walled ground-tissue. After this zone has begun to develope the formation of secondary cortex commences.

(7) Successive layers of periderm are formed, by which the whole of the primary cortex is eventually removed, the subsequent periderms arising in the outer part of the secondary cortex.

The occurrence of secondary thickening in this little group of Irideae, a group which is so narrowly limited both systematically and geographically, appears to us to be a fact of

considerable interest. It is impossible to doubt that secondary growth in the Irideae has originated *de novo*, and probably at a comparatively recent period, after the Order had attained something like its present development and geographical distribution.

In spite of this there is a remarkable general agreement between the process in Irideae, and that in the arborescent Liliaceae and in the Dioscoreae. But in these two groups also secondary growth must have started independently. We arrive then at the conclusion that a closely similar mode of anatomical development must have been separately evolved in at least three distinct groups of Monocotyledons—probably more. We thus find that the phenomena which we have considered in this paper offer a striking example of homoplastic modification, i. e. of the origination of similar, and apparently homologous structures in groups of organisms which are phylogenetically distinct.

It is very probable that the first origin of secondary growth may be taking place in some of the Monocotyledons at the present day, just as we find medullary bundles appearing in certain Dicotyledons as an individual peculiarity. From this point of view it would be very interesting to examine some of those species of *Aristea* which are not shrubby, and to see whether their short stems show any indications of secondary increase.

For our material we are indebted to the Director of the Royal Gardens, Kew; to Prof. F. O. Bower, F.R.S., Regius Professor of Botany in the University of Glasgow; and to Mr. F. W. Burbidge, F.L.S., of the Trinity College Botanic Gardens, Dublin, to all of whom we tender our warm thanks.

The investigation was chiefly carried on in the Huxley Laboratory for Biological Research, at the Royal College of Science, London; it was completed in the Jodrell Laboratory of the Royal Gardens, Kew.

EXPLANATION OF FIGURES IN PLATES III, IV, AND V.

Illustrating Messrs. Scott and Brebner's paper on Secondary Tissues in Monocotyledons.

Figs. 1–3. Development of tracheides.

Fig. 1. *Yucca, sp.* Young secondary bundle in tangential section; a developing tracheide, with its relatively large nucleus, is shown. × 133.

Fig. 2. *Yucca, sp.* Young tracheide isolated by maceration, from a developing secondary bundle. The cells to the left are desmogen-cells, for comparison with the tracheide, which has only reached about a quarter of the average mature length: at *a*, and perhaps at other points, a short branch is being formed. × 200.

Fig. 3. *Dracaena fragrans*, Gawl. Young tracheide and desmogen-cells as in last figure. Note that the nucleus is comparatively near one end of the tracheide—a common case. × 200.

Figs. 4–8. Roots of *Dracaena*.

Fig. 4. *Dracaena fragrans*, Gawl. Transverse section of large adventitious root, showing secondary thickening. On the right this has taken place entirely outside the endodermis; to the left it has gone on partly inside and partly outside. Portions of the ruptured endodermis are seen imbedded in the secondary tissues. × 17. *pd* = periderm, *c* = cortex, *cb* = cambium, t_2 = secondary tissues, *en* = endodermis, *pc* = pericycle, t_1 = primary tissues of central cylinder.

Fig. 5 Transverse section showing secondary thickening entirely outside the endodermis. In the secondary tissues, t_2, four concentric bundles are shown. *px*, one of the protoxylem-groups of the primary cylinder. Other lettering as before. × 117.

Fig. 6. Transverse section showing commencement of secondary growth in a transitional region. It is chiefly pericyclic, but a few divisions have taken place in the cortex. *en*, fragments of endodermis. Secondary bundles, derived from the pericyclic cambium, are in process of formation. ph_1, one of the peripheral phloëm-groups of the primary cylinder. × 117.

Fig. 7. Transverse section passing through part of the insertion of a branch-root. At the base of this is a large mass of secondary tissue, formed from pericyclic cambium. The endodermis is ruptured. To the right there has been no pericyclic thickening, but secondary growth has started in the cortex; b_2, developing bundle. Other lettering as before. The limit of the primary cylinder of the parent root is obvious, if traced from *pc* on the right. × 75.

Fig. 8. *D. Draco*, L. Transverse section showing an early stage of cortical thickening. Three or four tangential series of cortical cells are taking part in the cambial divisions. *r* = raphide-sac, marking inner limit of primary cortex at this

point. Other lettering as before. Only a few endodermal cells are thick-walled. × 175.

Figs. 9-17. *Aristea corymbosa*, Benth.

Fig. 9. Transverse section of young stem before secondary thickening. The light part is the central cylinder, with its scattered bundles. The dark zone is the cortex. On the right the section passes near the base of a leaf. Leaf-traces are seen passing through the cortex. × 17.

Fig. 10. Corresponding section through an old stem. *lf* = remains of leaf, *pd* = periderm, *l* = lenticel, *c* = cortex, *cb* = cambium, t_2 = secondary tissues, showing the two zones. t_1 = primary cylinder, outer part shaded to indicate sclerosis. All reference lines run to *outer* limit of zone indicated. × 17.

Fig. 11. Transverse section 2 mm. from apex. Outer part of cylinder; primary development in progress. Note *irregular* divisions of merismatic cells. The shaded cells in the cortex are tannin-sacs. *c* = cortex, *m* = primary merismatic zone or 'Thickening Ring,' b_1 = primary desmogen-strand. × 266 (reduced from × 400).

Fig. 12. Transverse section 5 mm. from apex. Primary merismatic divisions have ceased. Primary bundles shown in various stages. *px* = protoxylem of most advanced bundle shown. Other lettering as before. × 266 (reduced from × 400).

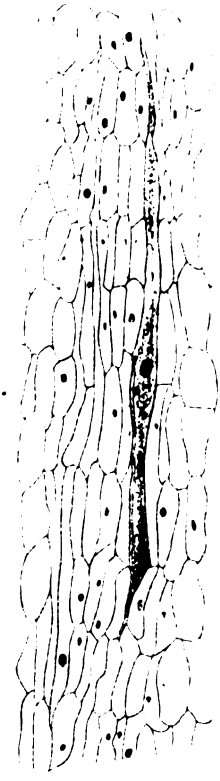
Fig. 13. Transverse section from older part of stem. Cambium in full activity. Note that divisions take place in more than one layer. Shading indicates probable limits of secondary tissue already formed. Primary parenchyma of cylinder has become sclerotic. *c* = cortex, *cb* = cambium. × 266 (reduced from × 400).

Fig. 14. Transverse section showing cambium and part of outer secondary zone from an old stem. Secondary bundles sharply defined. *pd* = periderm, c_1 = primary cortex, c_2 = secondary cortex, *cb* = cambium, b_2 = secondary bundles. × 117.

Fig. 15. Radial section through secondary tissues of a very old stem. No primary cortex. Periderm has been formed in secondary cortex. pd_2 = internal periderm, c_2 = secondary cortex, *cb* = cambium, b_2 = very young secondary bundle: x_2 = xylem, ph_2 = phloëm of mature bundle. × 117 (reduced from × 175).

Fig. 16. From a macerated preparation. *tr* = young tracheides, one still surrounded by desmogen-cells, the other free. Contents contracted by maceration. × 400.

Fig. 17. Complete mature tracheide, shown in two halves, macerated. The borders of the pits are not shown. × 200 (reduced from × 400).



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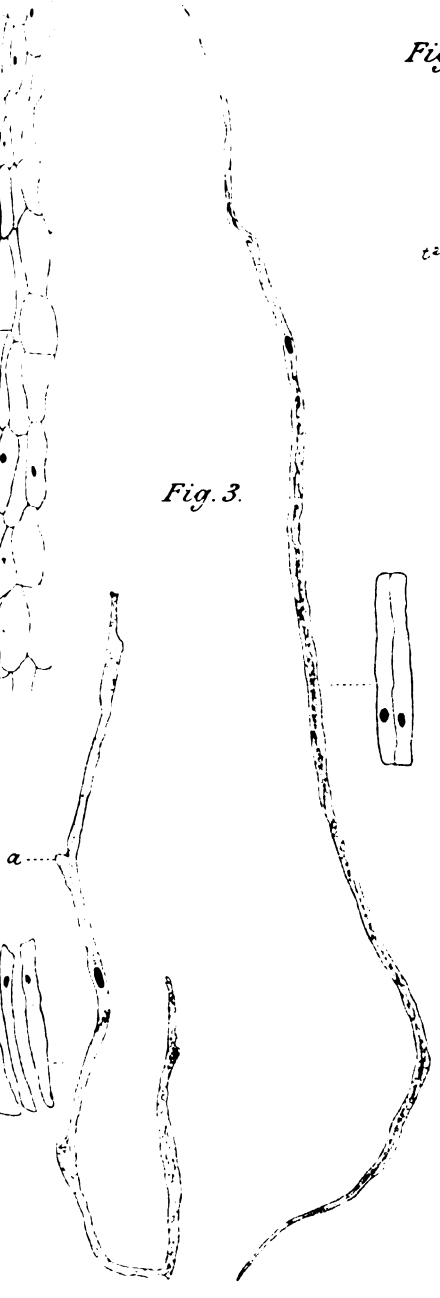

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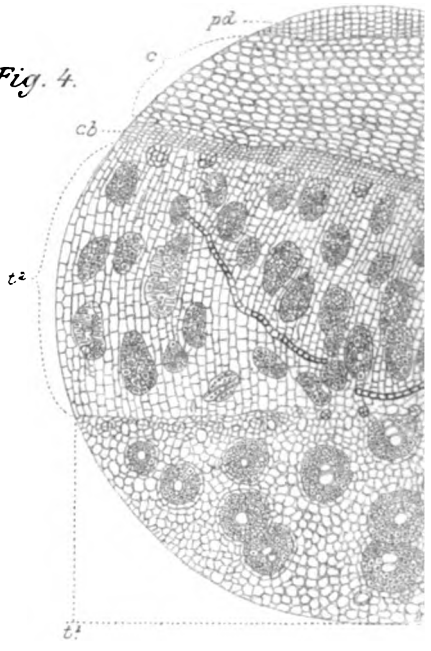

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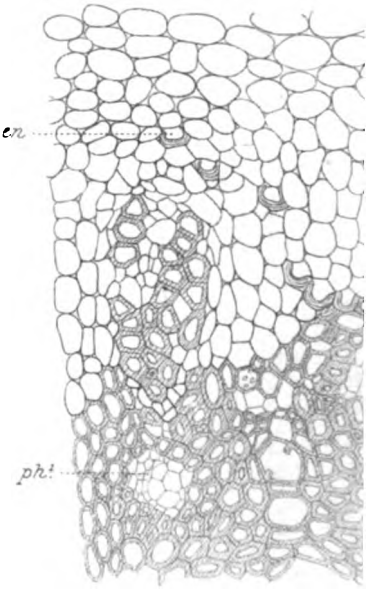

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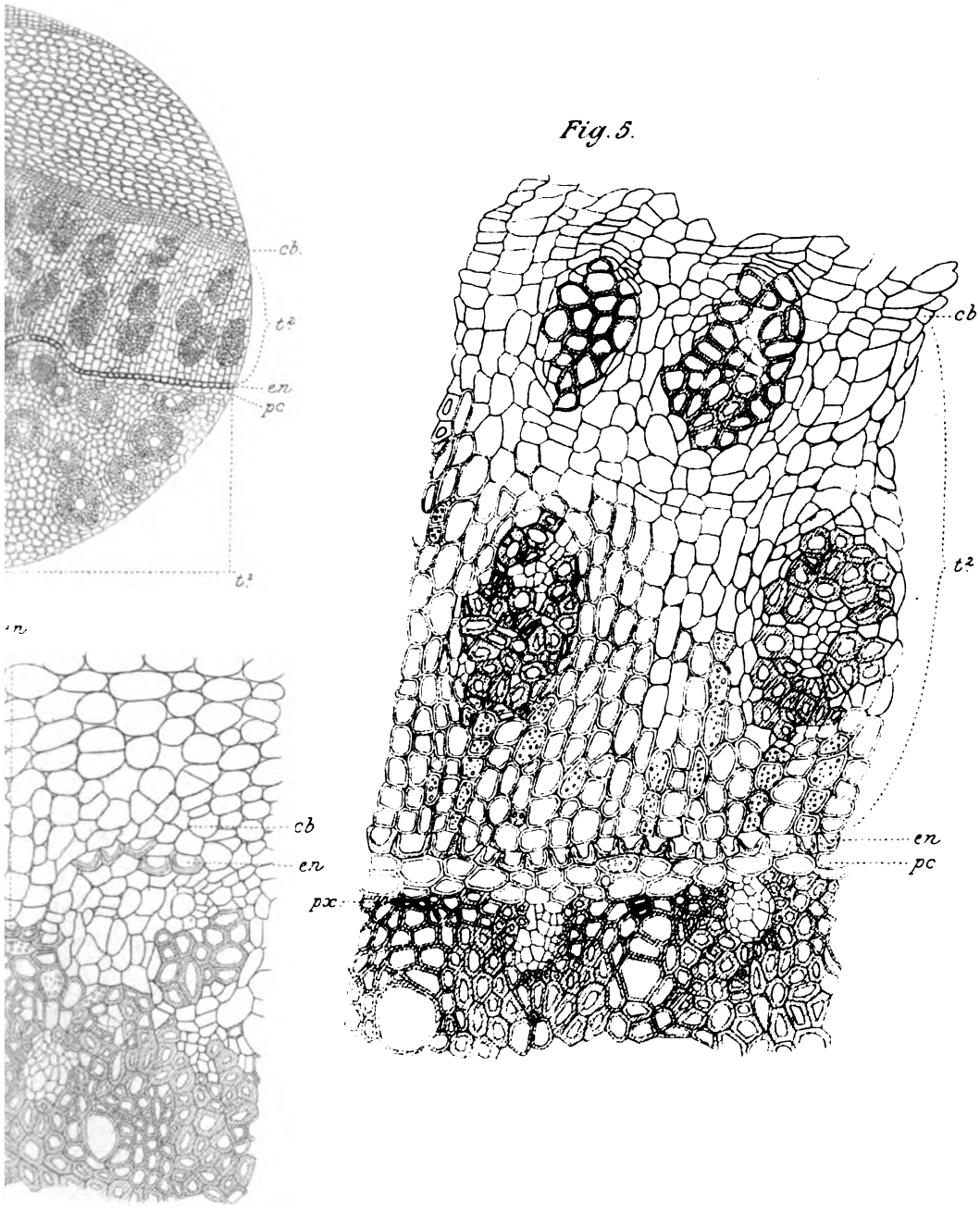

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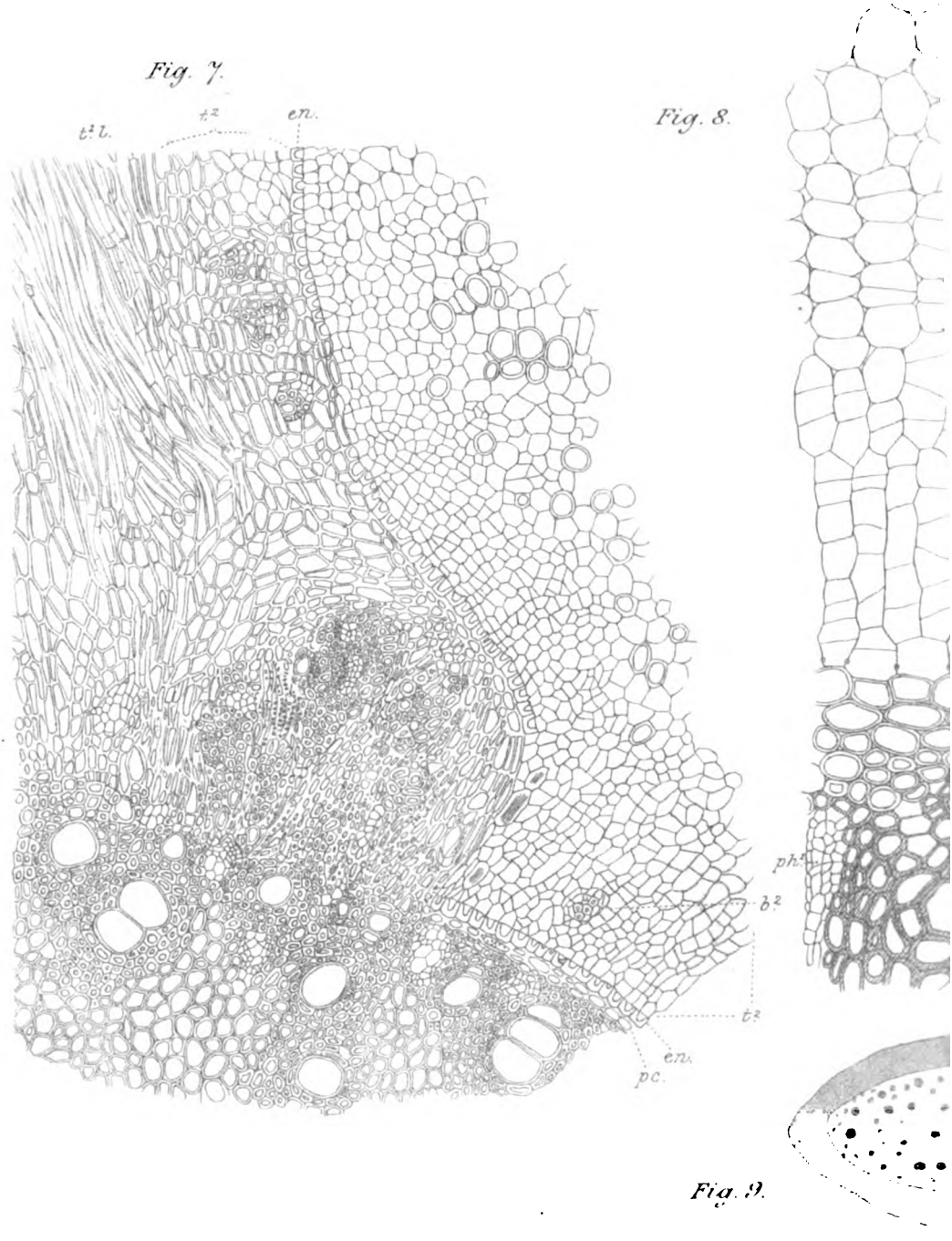

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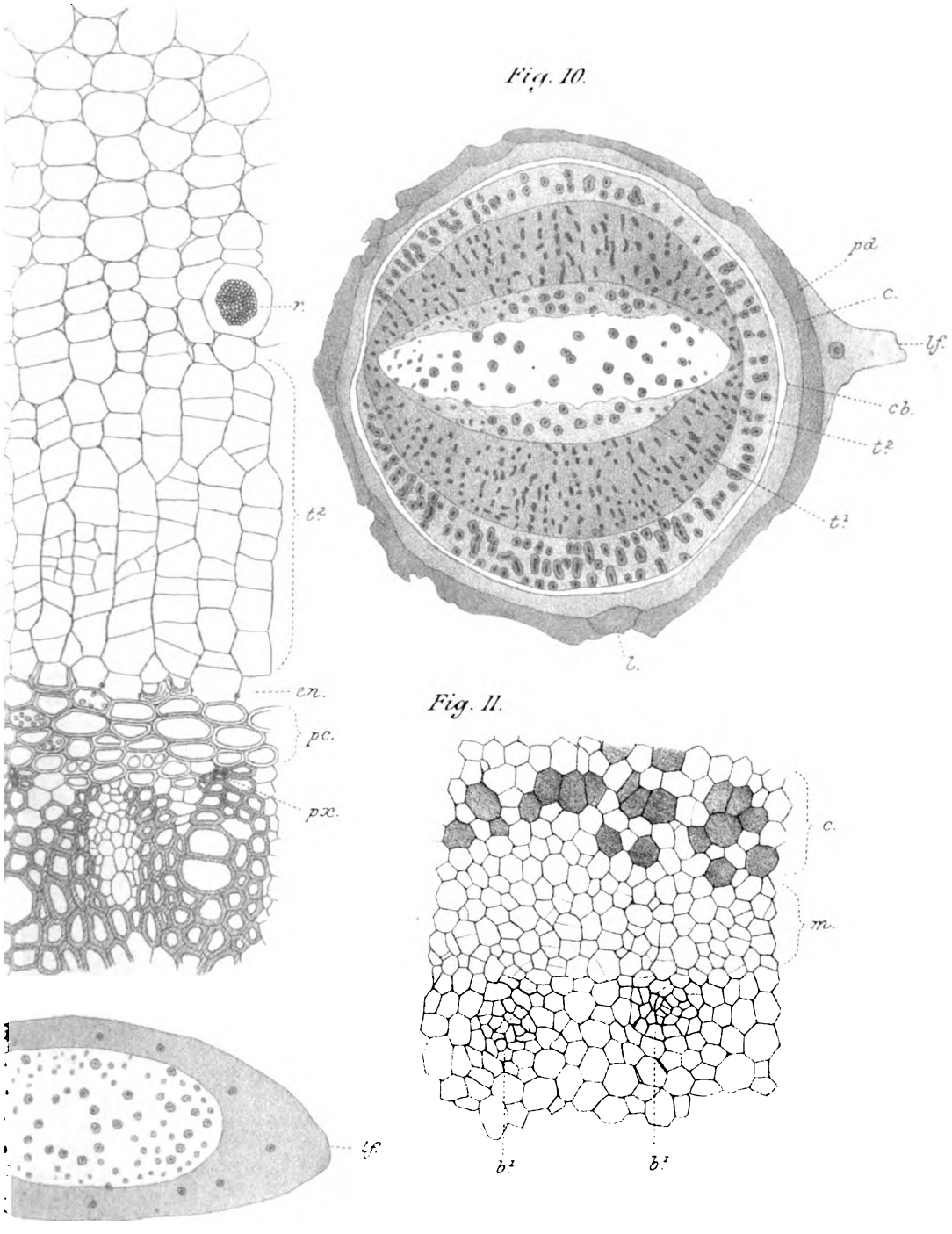

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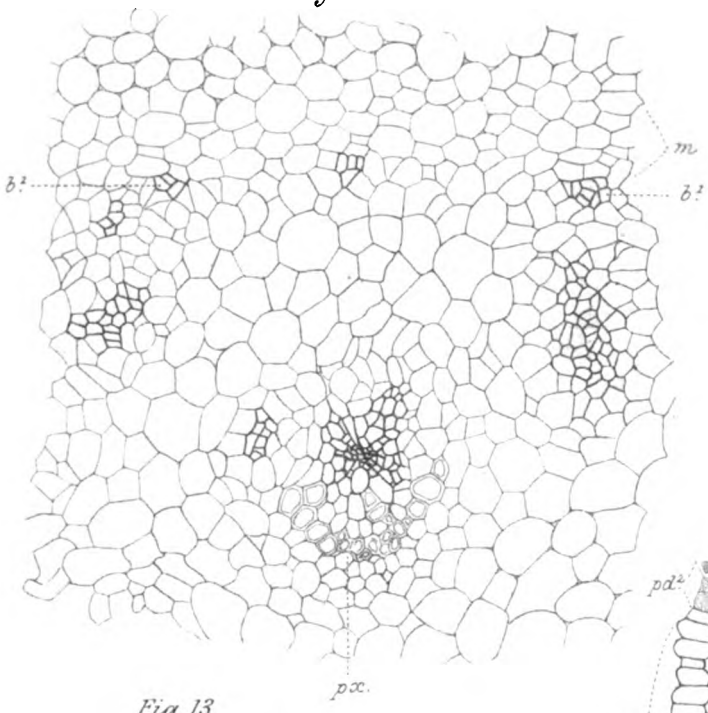

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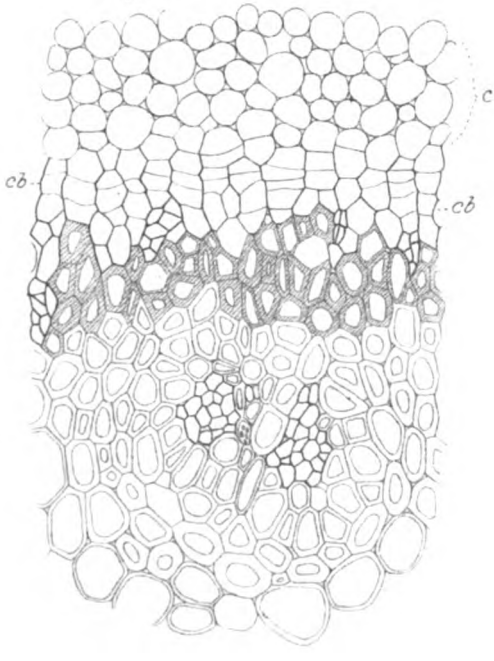

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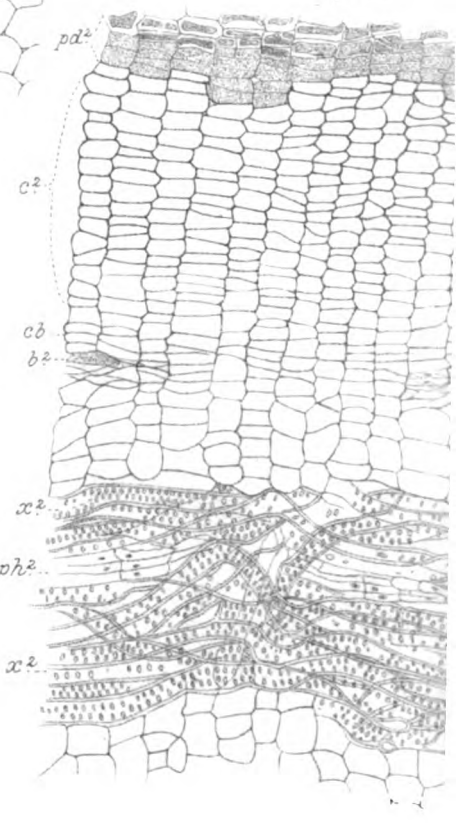

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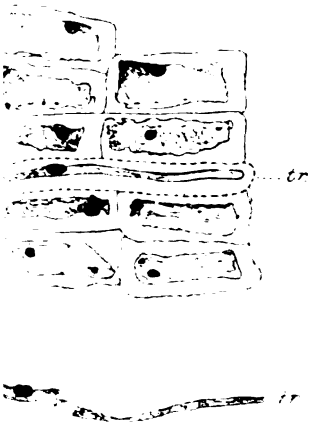

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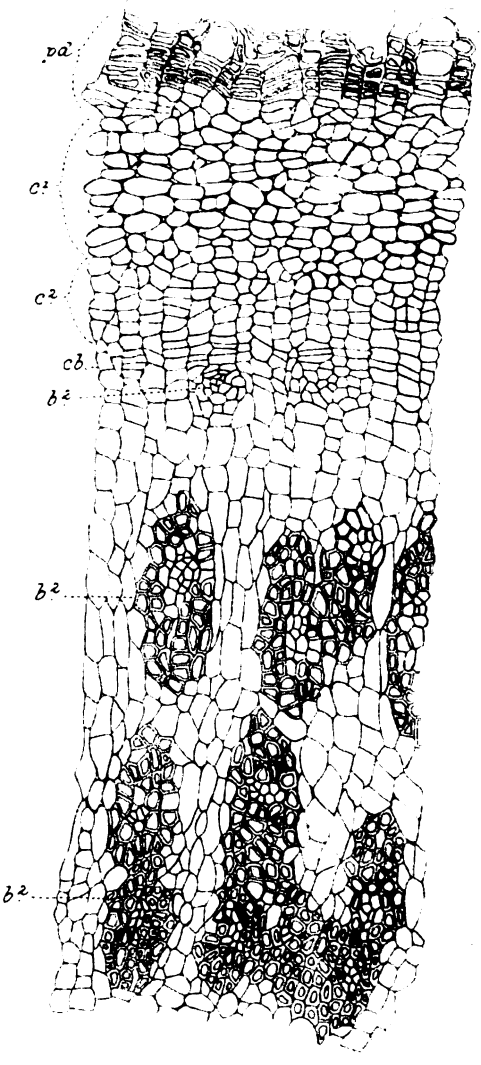

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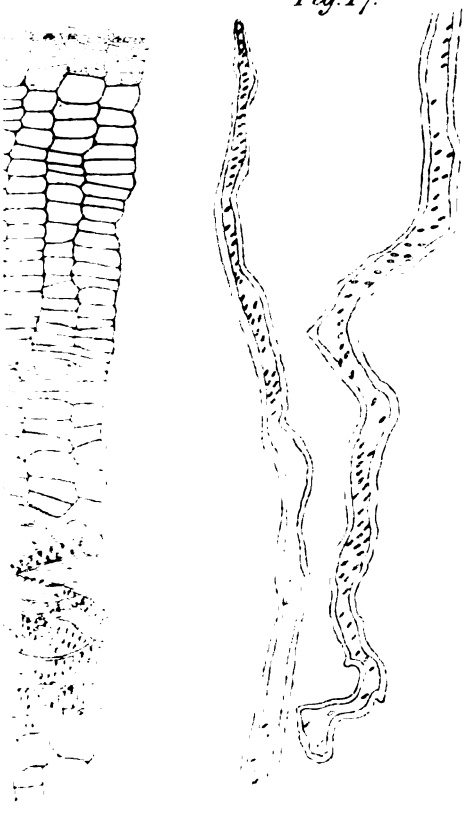

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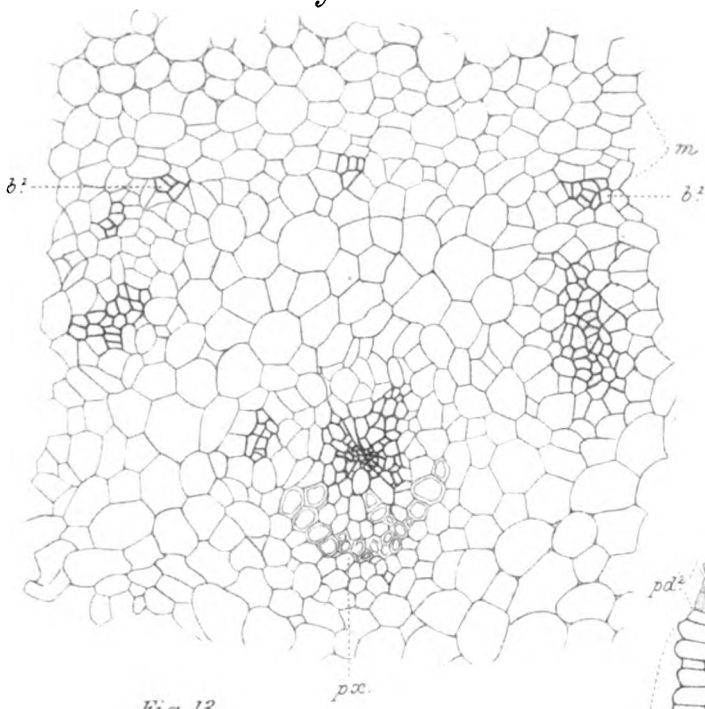

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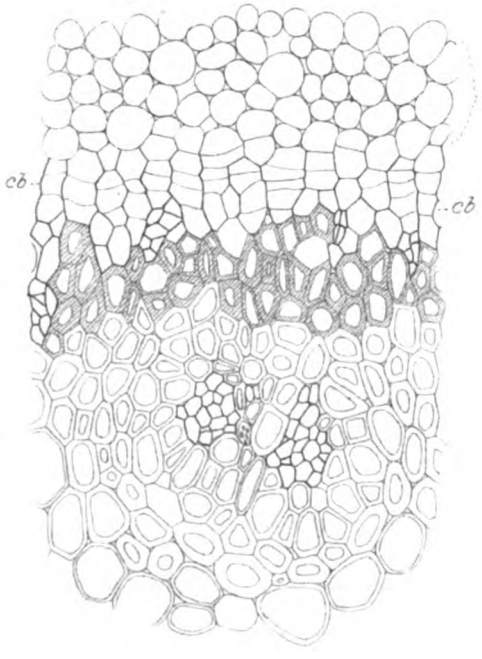

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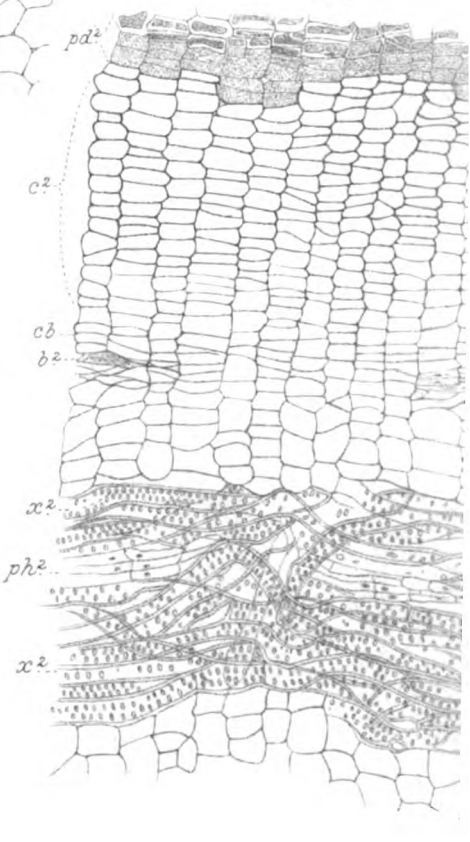

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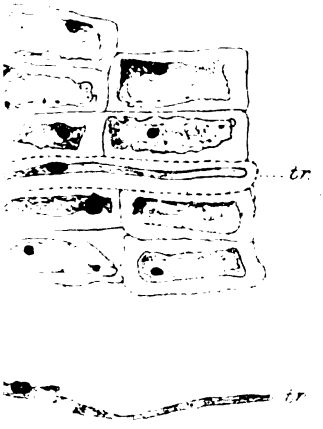

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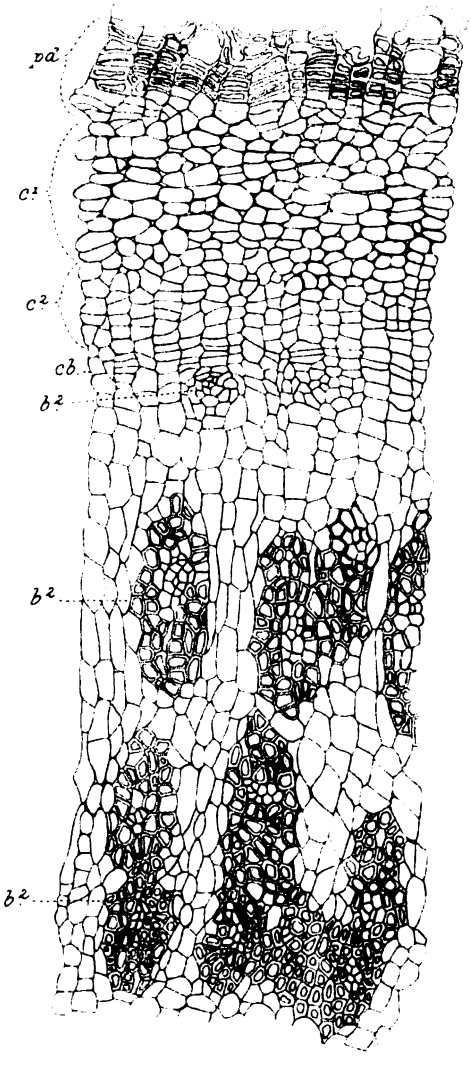

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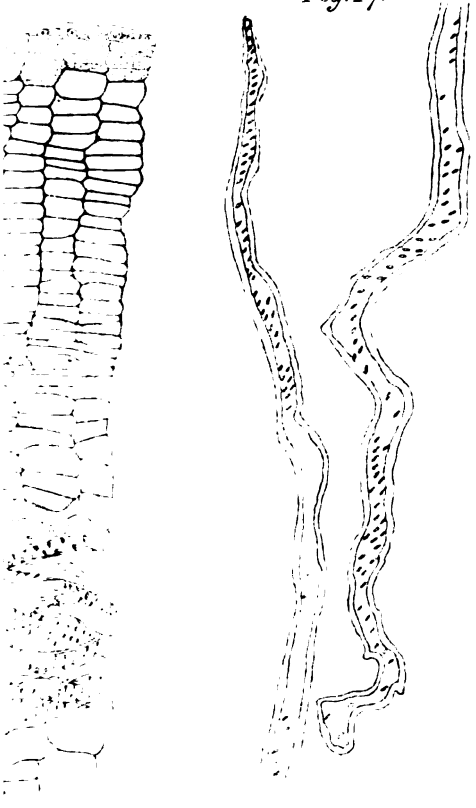

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On a Cambial Development in Equisetum.

BY

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Assistant to the Professor of Botany in the University of Glasgow.

With Plate VI.

THIS paper includes an investigation of a cambial development in modern Equisetaceae, an enquiry into the nature of certain features of Calamitae[1], and a reconsideration in the light of the results thus obtained of the debated question of the unity of the Calamitae as a group, and of their systematic position.

Regarding the problems connected with branching and infranodal canals, nothing will here be said; and the question of the nature of the reproductive organs will be mentioned only incidentally.

The main features of the Equisetaceae have been described in the works of Bischoff, Hofmeister, Thuret, Sanio, and Cramer, leading up to the monograph of Duval-Jouve and that of Milde. From the year in which Duval-Jouve's work was published till now, there have been frequent additions in detail to our knowledge of this group, the chief contributions on points of vegetative structure coming from Rees, Pfitzer, Nägeli and Leitgeb, Russow, Van Tieghem, Janczewski,

[1] The term *Calamitae* is here used, as by Solms-Laubach, to designate the stems and branches which, together with fructifications and other organs ascribed to them, constitute the group *Calamarieae*.

[Annals of Botany, Vol. VII. No. XXV. March, 1893.]

Famintzin, Sadebeck, Van Tieghem and Douliot, Müller, and Strasburger[1].

Thus this order, with its one genus and diagrammatic regularity of structure, has come to be regarded as one of the best understood groups of plants.

Buried in more recent strata are found fossils whose close relationship with modern Equisetaceae is not called in question; but the Carboniferous, Permian, and Triassic formations contain a group of fossils, the Calamitae, which some palaeobotanists would place bodily beside Equisetaceae, but which certain others seek to divide into two classes by removing one section and placing it among the Phanerogams.

The problem can best be approached by such a brief description of Calamitae showing minute structure, as is required for the purpose of this paper.

In a transverse section passing through an internode of one of these stems, there is seen a ring of woody wedges united by inter-fascicular tissue. The angle of each wedge is directed towards the centre of the stem, and each abuts on a cavity of its own.

Within this first ring is a second, composed of parenchymatous cells which form the outer limit of a central cavity.

Such is the appearance commonly presented in transverse section; but in a few cases a peripheral ring of the rind has also been preserved (see Williamson's[2] figure; and Plate VI, Fig. 11).

Tangential sections through the woody wedges show that these wedges represent strands passing longitudinally through the internodes. The strands belonging to adjacent internodes alternate—in most types—but are united into one continuous system; for each strand bifurcates in the node, so as to give an arm to each of the two strands lying to the right and left of it in the adjacent internode. Thus the commissures in the node form a zig-zag line similar to that found in the Equisetaceae.

[1] See list at end of paper.

[2] Phil. Trans., 1871, Plate XXIII, Fig. 9.

Median longitudinal sections show that the central cavity extends throughout the internodes, but is interrupted at the nodes by a disk of tissue which is sometimes entire, sometimes perforate. Williamson has shown that this canal existed during the life of the plant and was formed by absorption of the pith.

Such sections also show that the cavities seen in transverse section at the inner angle of the woody wedges, represent canals which follow the woody strands in their course through the internode. In the node these canals are sometimes interrupted, but sometimes bifurcate along with the woody strands and accompany them into the next internode. Thus the canals of adjacent internodes alternate, and may or may not be continuous.

Radial sections further show a point of much importance to this paper. The thickness of wood in the node exceeds that of the internode, so that the wood-strands project at the node with convex contours, centrally into the pith, and peripherally into the rind.

If the central cavity of such a specimen as has just been described were filled under pressure with material which would form a cast, the cast so produced would be cylindrical or somewhat conical, impressed at intervals with a circumferential groove corresponding with the nodal convex projections of the wood, and moulded into ridges and furrows, each furrow corresponding to the inner angle of a strand of wood. Sometimes all that remains to represent one of the Calamitae is such a cast covered, perhaps, by a thin layer of coal derived from the actual tissue of the plant now crushed and altered beyond recognition. This is the teaching of Williamson enforced more recently and very convincingly by Stur.

From this it may be inferred that there are three types of fossilization of Calamitae:—

(1) Petrifactions showing actual plant-structure.

(2) Mere casts invested by a thicker or thinner layer of coal.

(3) Mere moulds or impressions retaining perhaps a covering-layer of coal.

The casts were the first forms to attract attention and on them Suckow established his genus *Calamites.* Brongniart in 1828 adopted this genus and placed it side by side with *Equisetum* thus:—

Equisetacées (*Equiseteae*) { *Equisetum.* *Calamites.*

This represents the view then generally accepted; for the comparison of a Calamite with a reed had soon been relinquished, and only the etymology of the name suggested phanerogamic affinities.

The discovery of petrifactions showing minute structure at once introduced discord. The difference of opinion and nomenclature is shown in the following table:—

CALAMITAE.

Calamites (Suckow)	*Calamites*	*Calamites*	*Calamites* { *Calamites* (Calamiteae)	Allied to Equisetaceae
All allied to Equisetaceae	*Calamitea striata* (Cotta) *Calamitea bistriata*	*Calamodendron striatum* (Brongniart) *Calamodendron bistriatum*	*Calamodendron striatum* *Arthropitys bistriata* (Göppert) } Calamodendrées (Calamodendreae)	Gymnospermous
Williamson		Brongniart	Grand 'Eury	

Cotta had established a genus *Calamitea* which included *C. striata* and *C. bistriata* (and, it would seem, also some Conifers). *C. striata* was described by Unger in Petzholdt's work. Secondary thickening is obvious in this plant, and Brongniart having regard to this fact, removed Cotta's genus

to a place among the Gymnosperms; and emphasized this change by altering the name *Calamitea* to *Calamodendron.* He thus asserted the existence of two genera, one to be placed side by side with modern Equisetaceae, the other to be included among the Gymnosperms.

Göppert gave generic value to Cotta's two species calling them *Calamodendron striatum* and *Arthropitys bistriata.*

Grand 'Eury accepted the change, thus arriving at the classification shown in the last column of the foregoing table which represents the view held by what has been termed the French school, headed by Brongniart, Grand 'Eury and Renault, together with Schenk.

The group Calamodendrées (Calamodendreae) was thus understood to consist of Calamitae which show secondary thickening; together with the casts and impressions referable to such forms.

The name *Calamites* (Calamiteae) was reserved for such Calamitae as may be shown to have no secondary thickening; together with casts and impressions referable to such forms.

There is however no evidence to show that Calamitae devoid of secondary thickening ever existed All specimens which show minute structure exhibit such thickening. Schenk proved that in certain remains from which secondary thickening was held to be absent, the supposed cortex was really secondary wood.

Grand 'Eury himself has recently admitted that in the cases of *Calamites cannaeformis* and *Calamites varians* (forms considered by the French school as referable to the class devoid of secondary thickening), we have casts derived from a type which shows secondary thickening, namely, *Arthropitys*[1].

Under the circumstances we may now limit our attention to consideration of such forms as possess secondary thickening, that is, to all Calamitae of whose minute structure we have any record.

Williamson's paper, in the Memoirs of the Manchester

[1] Comptes Rendus, 1886, p. 394.

Literary and Philosophical Society[1], leaves little doubt that such forms constitute one group of closely related plants. He distinguishes three types: to these may be added a fourth, described by Dawson[2], which completes a very closely-connected series. The relationship of these forms is shown in the following table:—

	Woody Wedges	Interfascicular Tissue
(*a*) *Calamites*	Elements barred; thin medullary rays.	Cellular
(*b*) *Calamopitus*	Elements reticulated on radial walls; thin medullary rays.	Cells elongated; medullary rays.
(*c*) *Calamodendron*	Elements barred; medullary rays more massive than in (*a*) or (*b*).	Prosenchymatous and partly selerenchymatous; medullary rays more massive.
(*d*) *Eucalamodendron*	'True bordered pits or pseudo-scalariform slit-pored tissue.'	

The distinguishing features of these four types are found in tissues formed in the course of secondary thickening. The barring of the elements is in all essential points identical with annular thickening. Williamson writes[3]—'In several instances I have noticed that the vessels at the inner extremity of the wedge were barred, whilst those constituting its peripheral portion were reticulated.' In fact the differences between the four types are such as would be recapitulated during the development of *Eucalamodendron*, the most highly differentiated of the series. It is difficult to avoid correlating such differences with differences of bulk attained by the stems; and on turning to Dawson we find 'that under (*a*) and (*b*) there are some species in which the woody cylinder is very thin in comparison to the size of the

[1] Memoirs of the Manchester Literary and Philosophical Society, Third Series, vol. X, 1886–87.

[2] Geological History of Plants, London, 1888.

[3] Phil. Trans., 1871, p. 481.

stem. In (*c*) and (*d*) the woody cylinder is thick and massive and the stems are often large and nodose.'

Williamson regards (*c*) in the foregoing table as the *Calamodendron* of Brongniart: Dawson (*b*). That such difference of opinion is possible, proves how very closely related are the two types. The four types form a series in which (*d*) stands in the same relation to (*c*) as (*b*) does to (*a*).

Having regard to all these facts we conclude with Williamson that we are dealing with a group of plants which are closely allied to one another.

We are thus brought to the consideration of the problem regarding the systematic position of this group.

The removal by Brongniart of the forms in question to a place among the Gymnosperms was the outcome of his assumption that a plant which showed secondary thickening could not be a Cryptogam.

The following considerations lead me to regard this assumption as unjustifiable:—

(1) The vascular and reproductive systems are not so intimately connected that we can infer that because one plant has open bundles, and another plant closed bundles, therefore their reproductive organs will be essentially different: nor can we infer that because in two given plants the bundles are open, therefore their reproductive organs will be similar.

(2) Cambial activity is found in plants whose affinities must be very remote, for example, in *Quercus* and in *Laminaria.*

(3) While normally absent from a class (Monocotyledons) secondary thickening may be present in some members of it (certain Liliaceae).

(4) In a class which normally possesses secondary thickening, it may be present in one species, and practically absent in another of the same genus (*Ranunculus fluitans*).

(5) Among extinct plants also secondary thickening is known to have been absent from a species of a genus possessing it (*Lepidodendron Harcourtii*).

(6) Further, secondary thickening existed in ancient crypto-

gamic forms though absent in their nearest modern representatives (present in *Lepidodendron*, absent in *Selaginella*).

(7) Secondary thickening is not unknown in Vascular Cryptogams of the present day. It is recognized in *Isoëtes*; and it has also been described in *Botrychium*. Solms-Laubach[1] considers that in *Botrychium* the point requires to be cleared up by further investigation; for the present we defer such examination. The objection that such secondary thickening is slight, is invalid, for mere questions of size give no sure basis for scientific classification.

Thus we conclude that secondary thickening must sometimes be homoplastic rather than homogenetic; and consequently that, even in the absence of a cambial activity in modern Equisetaceae, it would be unwarrantable to deny the possibility of affinity between them and Calamitae with secondary thickening.

While this is true, it is well that the Equisetaceae should be re-examined with a view to discovering whether cambial activity is really quite absent. This paper originated in a suggestion made by Professor Bower, with full appreciation of its palaeobotanical bearing, that in *Equisetum* some slight cambial activity might be found at the nodes, where, as is well known, the wood is more extensive than in the internodes; sections cut there and then gave indications which provoked more deliberate investigation.

Williamson[2], as the result of his examination of *Equisetum maximum*, brings forward certain differences of structure which render it undesirable to unite *Calamites*, with Equisetaceae 'though there are some points of resemblance between the two plants that sorely tempt a botanist to do so.' We seek to discover by a re-examination of this species and of one of the Calamitae, how far such differences exist, and to estimate roughly what may be their systematic value.

Fig. 1 is a diagram from a camera lucida outline of a radial section of *Equisetum maximum*. The section passed through

[1] Fossil Botany, Eng. Ed., p. 223. [2] loc. cit. p. 503.

the centre of a bundle in the lower internode. Therefore it shows the carinal canal of the lower internode with the remains of the protoxylem; the nodal portion of a bundle; the bundle belonging to the leaf. For the same reason it does not show either of the two side-strands of xylem seen in transverse section of the mature internode (*g*, *g'*, Fig. 5); the bundle-strands contributed to the branches; nor the bundles of the upper internode; for the bifurcation has carried them out of the plane of section. With this is to be compared Fig. 2, a corresponding diagram from a transverse section passing through a node and cutting two bundles about their points of bifurcation. The branch-bundles (*bb*) and the leaf-bundles (*lb*) are seen passing out almost at the level of the bifurcation.

The strong development of wood in the node, as seen in Figs. 1 and 2, at once attracts attention, so marked is its contrast with the feeble development in the internodes as seen in Figs. 1 and 5. The greater bulk of wood in the node is a well-known feature (see Duval-Jouve[1] and recently Strasburger[2]), but it appears that no attempt has hitherto been made to trace its development or to estimate its significance.

Fig. 3 is from a transverse section through this mass of wood in a mature node of *Equisetum maximum*. The cells of the wood and of the bast, towards the middle of the bundle, are seen to be arranged in radial rows. Fig. 4 shows two such rows of cells more highly magnified. Now this regularity is not noticeable among the cells either of the wood nearer to the centre of the stem, or of the bast nearer to the periphery; and the whole arrangement suggests that a plate of tissue has been intercalated between two older growths by the activity of a cambium-like meristem.

Examination of radial (Fig. 9) and tangential (Fig. 8) sections, strengthens this opinion. We see there cells similar to those of an ordinary cambium; and a cell-formation re-

[1] Duval-Jouve, Hist. nat. des Equisetum, Pl. VIII, Fig. 10.

[2] Strasburger, Ueber den Bau und die Verrichtungen der Leitungsbahnen, p. 433.

sulting from their division which is similar to that in an ordinary secondary thickening.

Corroborative evidence is obtained by tracing the development of the vascular system. As is well known, each pseudo-whorl of segments derived from the tetrahedral cell of the *punctum vegetationis* produces a node with a leaf-sheath, and the internode beneath.

At first there is no differentiation of the internode; its development is the result of an intercalary growth at the base of the tissue produced from one of the pseudo-whorls.

The course of the bundles is early marked out by the differentiation in the bud of the annular protoxylem of the stem, and of the leaf-bundles. Afterwards, during the elongation and general growth of the internodes, the protoxylem-elements are separated and destroyed, giving rise to the carinal canals. Only protoxylem is lost in the formation of these canals. There is no appearance of destruction of other tissues; and indeed the position of remains of protoxylem on the outer borders of the canals proves that the bundles have lost no other elements. Thus, in the mature internode, the only tissue missing from the bundle is the protoxylem.

Now, on comparing a transverse section through an internode of the bud (Fig. 7) with a transverse section through a mature internode (Fig. 5), it is seen that the number of cells in the radial thickness of the bundle is in both cases about equal. Hence it may be concluded that tangential cell-division is early arrested in the internodes.

In the nodes on the contrary this is not so. Fig. 6 is from a transverse section through the node adjacent to the immature internode from which Fig. 7 was drawn. With this is to be contrasted Fig. 3, drawn from a corresponding section of a mature node. In the immature node there are fewer cells in the radial thickness of the bundle than in the mature node; as yet the annular protoxylem is the only part of the wood fully developed, for the cell-division which produces the radial rows in which reticulate xylem is developed, has only recently begun.

Hence, if it be argued that the structure in question is primary and in no way comparable with a secondary thickening, it may be answered: that after the bundle has attained in the internode its full number of cells in radial thickness, and after tangential division in the corresponding tissues of the node has ceased, a plate of tissue has been intercalated between the protoxylem and protophloëm of the node; that the xylem thus formed is mostly reticulately thickened, whereas the thickening of the protoxylem is annular; and that the intercalation has been accomplished by the activity of a meristem whose cells are cambiform.

Further, there are recognized cases in which the transition from primary to secondary tissue is immediate and the distinction between them scarcely observable. *Ranunculus repens* is an example of this [1], and still more striking in this respect is *Ranunculus fluitans* where, in correlation with aquatic habit, the vascular system is weak alike in its primary and in its secondary development—so far as such secondary development exists. Indeed, were it not for its systematic position, such a plant as *Ranunculus fluitans* would scarcely be recognized as possessing secondary thickening; and in the absence of preconceived ideas, Fig. 3 would be considered a much more typical illustration of such formations.

The fact that in the node of *Equisetum* the thickening extends only a short distance longitudinally cannot be held to weaken the argument: it is a question of secondary growth in *thickness*.

But whether the name, secondary thickening, is accorded to this process or not, matters very little for our present purpose, as it can be shown that a cambial activity exists, essentially similar to what is found in Calamitae, only less in extent.

First, let it be noted that in the young internode (Fig. 7) the side-groups of xylem *gg'* seen in Fig. 5 are not yet developed. They are differentiated during the basal intercalation of the internode previously mentioned, and after the

[1] De Bary, Comp. Anat. Fig. 152 and Fig. 153.

process of development in the node, as illustrated in Fig. 6, has continued for some time.

Thus it is seen that in the Equisetaceae the development of wood later than protoxylem begins in the node, and afterwards extends through the internode.

We now proceed, to examine certain inferences which have been drawn from the known structure of Calamitae, to adduce evidence, and to draw conclusions as to the course of development of the vascular system in these plants for purposes of comparison with what we have seen in Equisetaceae.

Fig. 11, from a transverse section of a young Calamite, shows a bundle and part of the interfascicular tissue, as also a portion of the rind with the cambial layer crushed and distorted, but still recognizable, and showing its connection with the inner tissues.

There is some difference of opinion as to the nature of the canal found at the inner angle of each woody wedge. Regarding these canals, Williamson writes[1]: 'Mr. Binney . . . . . was doubtful respecting their nature. He says that Dr. Hooker, "after carefully examining these openings, I believe, came to the conclusion that they were passes for a peculiar kind of tissue which has unfortunately been destroyed, rather than the mere cavities which we now see in the specimens." This supposition, however, is certainly not correct.'

Schenk, who would place the Calamitae in question among the Gymnosperms, gives a figure[2] in which phloëm takes the place of the cavity, and the adjacent medullary tissue is described as xylem; thus the cambium and the tissue formed from it (Fig. 11) would have to be considered as extrafascicular. Solms-Laubach[3] has seen the original preparations, and asserts that they are badly preserved and do not justify the explanations given; and to his view Schenk has given assent[4].

Fig. 10 represents another part of the same preparation from which Fig. 11 was drawn, and shows the remains of the protoxylem occupying the canal. The rings marked × are

[1] loc. cit. p. 485. [2] Zittel, Handbuch der Paläontologie, p. 237.
[3] Fossil Botany, Eng. Ed., p. 297. [4] Handbuch der Botanik, vol. IV, p. 109.

seen on focussing. While the nature of the isolated ring in Fig. 11 is not beyond suspicion, no doubt can be entertained as to the contents of the lacuna in Fig. 10. Thus it may be inferred that these canals, like the carinal canals of *Equisetum*, orginate in the destruction of protoxylem; and the accuracy of this conclusion is not affected by the fact that in neither of the two plants did the protoxylem ever occupy the whole length and breadth of a canal. To this extent it is true that the canal is due to the destruction of a tissue, but this tissue was lost, not after death, but during the development of the plant, and was xylem, not phloëm.

The projections of the xylem in the nodes, previously referred to, acquire significance in tracing the further development of the vascular system of Calamitae.

Williamson[1] writes as follows regarding the structure of the wood as seen in a vertical section of a mature stem :—

'The first feature which arrests attention in the vertical section is the material transverse enlargement of the woody zone which takes place at the node. This enlargement is both internal and external. In the former case the woody layer encroaches on the pith, and in the latter upon the bark. The increment is due to the development of a considerable number of barred or reticulated vessels, but especially the former, which take their rise in contact with the outermost medullary cells above the node, and following an arching course across it, their concavities being directed towards the medulla, again terminate as they arose from the medullary cells above the node, in those below it. It follows from this arrangement that only the outermost of these nodal vessels are prolonged across the internodes to the adjacent nodes above and below.

'In transverse section we find, as the vertical one would lead us to expect, that the woody wedges at the node are much longer from their medullary to their cortical surfaces than at the internodes, The canals from which they respectively take their rise are either wholly wanting here or are so reduced as to become quite inconspicuous.'

[1] loc. cit. p. 483.

Now the persistent wood of the Calamitae is almost wholly in radial rows, and is therefore regarded as secondary, the protoxylem having been almost entirely lost in the formation of the canals. Further, the position of the cambium in Fig. 11 shows that the development of the wood is centrifugal. It follows that the inner portion of the wood, which is found only in the node, was developed before that which is common to both node and internode.

The same considerations, reinforced by an examination of Williamson's figures, makes it seem highly probable that secondary thickening began before the elongation of the internodes, proceeded simultaneously with that elongation, and continued after the internodes had attained their maximum length. Now it was shown that the formation we have seen to result from cambial activity in the node of *Equisetum* originates in the bud, and that the side-groups of xylem in the internode are formed later during the elongation of the internode. If, then, our interpretation of the development of the wood in Calamitae is correct, the two cases are similar.

But whether or not it be accepted that secondary thickening of Calamitae began in the bud, this much is certain, that in its main features the course of development of wood of Calamitae was similar to that of *Equisetum*; the differentiation of the later wood began in the nodes and afterwards extended to the internodes. This conclusion is not drawn from hypothetical considerations, but is the logical inference from the results of observations made by Williamson unbiassed by any theory.

If we imagine the cambial development of *Equisetum* extended through the internodes, and also become interfascicular, we produce a picture of the state of affairs in Calamitae.

In the Equisetaceae, in correlation with their more or less aquatic habit, the xylem is greatly reduced; its mechanical function is performed in part by the collenchyma, and its duties as a water-carrier and storer are undertaken in great measure by the carinal canals, assisted sometimes by the central canals[1]. The carinal canals, however, do not extend

[1] Strasburger, loc. cit. p. 438.

through the node, and the presence there of the greater mass of wood is of importance in transmitting water contained in the canals, for the number of the elements compensates for the absence of an open channel. Thus it is seen that the secondary thickening existent in the node is of physiological importance apart from questions as to whether it may be incipient or persistent.

Returning now to the question of the affinity of the Calamitae, we have seen that the canal at the inner angle of each woody wedge does not represent lost phloëm, but is the same in origin as the carinal canal of Equisetaceae. That these canals in Calamitae are sometimes continuous from one internode to another, is a difference unimportant in itself and rendered still less important by the fact that Calamitae differ among themselves in this respect.

Further, we have shown that the formation of secondary wood in Calamitae is a point of resemblance rather than of mere contrast between this group and the Equisetaceae, the course of development being similar though unequal in extent.

Certain distinctions, previously referred to, drawn by Williamson[1] between Calamitae and Equisetaceae, amount to nothing more than amplified illustration of the fact already admitted, that cambial development has taken place to a greater extent in one case than in the other. Thus he points out in Calamitae the similarity between the wood of the node and that of the internode, and contrasts with this the abrupt transition in *Equisetum* from the annular elements of the internode to the reticulate elements of the node. But it is clear that more extensive thickening in *Equisetum* would have obliterated this difference.

Similarly it is remarked that there is nothing corresponding to the 'muriform' tissue of the 'primary medullary rays' of Calamitae. Williamson's drawings and description makes it certain that the bulk of the tissue to which he gives the name 'primary medullary ray' is a structure produced by an inter-

[1] loc. cit.

fascicular cambium (Fig. 11). He remarks that 'the cells of the pith become more regular in disposition' as we pass into the primary medullary ray; and again notes the suddenness of transition from pith-cells to 'primary medullary ray' cells seen in longitudinal section. As cambial activity in *Equisetum* has not extended to the formation of an interfascicular cambium, we can sufficiently account for the absence of such muriform tissue. However, as a matter of fact, cells which might be described as 'muriform' are found in the primary medullary rays of *Equisetum maximum*.

Certain others of the distinctions drawn are not involved in secondary thickening.

Williamson writes: 'We further discover in the mode of *Equisetum* that, in addition to the cellular diaphragm or extension of the pith that stretches across the fistular cavity, a still more dense layer exists, not only within the diaphragm, but which, as shown in Fig. 41, is continued in a direct line across both the vascular and cortical zones . . . . this dense layer truncates the vascular masses.'

Fig. 1 is a diagrammatic representation of the tissues in the node of *Equisetum maximum* — the species examined by Williamson. As might be expected, the cells of the node are little elongated except in the vascular system; but there is a gradual transition to greater elongation in the internode. In Fig. 1 there is an attempt to indicate relative shortness of cells in the parenchymatous portion by means of greater depth of shading. The shorter cells must, we conclude, be the 'denser layer' referred to by Williamson; but the layer, such as it is, cannot be described as 'intersecting the course of the vessels.' The vascular system passes, so to speak, without interruption through the layer.

No great importance can be attached to the distinction between Calamitae and Equisetaceae, which is based on more complete absorption of the pith and consequent continuity of the central cavities of certain Calamitae. Diaphragms are not always absent from the nodes; they are sometimes thick throughout, and sometimes thin down towards the centre.

Further, in *Equisetum maximum* the absorption of the pith is absent in the rhizome, partial in sterile portions of the aerial branches, and complete in the sporangiferous portions. Thus absorption of the diaphragms is not always found in Calamitae, and is not unknown in Equisetaceae.

Neither can great importance be attached to the fact that the leaves of Calamitae are free, while those of Equisetaceae are united into a sheath. The leaf is known to be the most plastic of organs, varying in form in even closely allied species; and the amount of cohesion of foliar organs may vary in a single plant, as is the case with the stipules of certain Stellatae, where the stipules of opposite leaves may remain perfectly free, or be free only towards their tips, or cohere entirely. Thus no great systematic importance could, under any circumstances, be attached to the cohesion of the leaves of Equisetaceae; still less, when the cohesion may be correlated with the weak development of the wood of *Equisetum* as compared with that of Calamitae. The leaf-sheath, by its peculiar structure [1], is fitted to give some slight support to the stem of an *Equisetum* which would be superfluous to the woody stem of a Calamite; and expanse of leaf is less important in the case of a distinctly herbaceous stem.

The foregoing conclusions convince us that Williamson was more than justified in maintaining, as he so long has done, that the presence of secondary thickening is no sufficient reason for removing any of the Calamitae from among the Vascular Cryptogams; and lead us to think that these plants resembled Equisetaceae in their vegetative organs even more than Williamson admits, inasmuch as certain distinctions which he has drawn disappear on closer investigation.

Renault, in maintaining his contention that these plants are gymnospermous, assigns to them certain sporangiferous spikes whose spores he calls pollen-grains. But in the absence of any proof that such spores produced pollen-tubes, it would be unwarrantable to apply to them any more definite term than

[1] Müller, Ueber den Bau der Commissuren der Equisetenscheiden, Jahrb. für wiss. Bot., 1888.

microspore. Nothing short of the discovery of undoubted seeds or germinated pollen-grains, in organic connection with these plants, would justify the assertion that they were spermaphytes or siphonogams; and even granting them proved seed-bearing plants, their vegetative characters would then be so unique that, having regard to the result of Treub's investigation of *Casuarina*[1], it would be rash in the absence of such developmental evidence to include them in any known group of Spermaphytes.

The results thus arrived at may be summarized as follows:—

(1) A cambial activity exists in the nodes of modern Equisetaceae.

(2) There is no evidence that secondary thickening was actually absent from any of the Calamitae.

(3) The types of Calamitae whose structure is known, form a very closely-connected series in which the distinctions found in secondary tissues are such as might well be correlated with difference in bulk; whatever be the systematic position of the Calamitae, they appear to form a united group.

(4) The canal at the inner angle of each woody wedge of some Calamitae originates in the destruction of protoxylem, and is not due to loss of the phloëm; consequently it has the same origin as the carinal canals of Equisetaceae.

(5) Cambial activity in Calamitae began in the nodes and thereafter extended to the internodes. In the nodes of living species of *Equisetum* a similar cambial activity is seen, which is less extensive and does not reach into the internodes.

(6) Thus cambial activity in Equisetaceae and Calamitae is the same in essence, but different in extent.

(7) Consequently the vegetative organs of the Calamitae present features which resemble those of the Equisetaceae more closely than has been admitted, while by this correspondence of structure the argument for classing Calamitae which show secondary thickening, among the Phanerogams, is effectually answered.

[1] Sur les Casuarinées et leur place dans le Système Naturel, Annales du Jardin Botanique de Buitenzorg, 1891.

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WILLIAMSON, Memoirs of the Manchester Philosophical and Literary Society. Third Series. Vol. X, 1886–87.

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EXPLANATION OF FIGURES IN PLATE VI,

Illustrating Mr. Cormack's paper on a Cambial Development in *Equisetum*.

REFERENCE LETTERS.

bb, branch-bundle.
cc, carinal canal.
d, the 'denser layer.'
end., endodermis.
g, *g'*, side groups of xylem.
l, leaf.
lb, leaf-bundle.
phl., phloëm.
r, protoxylem.
t, tannin.
vc, vallecular canal.
xy, xylem.
×, seen in a different focal plane.

Fig. 1. Diagram from a radial section through a node and portions of two internodes of *E. maximum*. The plane of section passes through the middle of the carinal canal and vascular bundle of the lower internode. The darkest shading represents the wood. In the ground-tissue, darkness of shading represents relative shortness of the parenchymatous cells. (× 10.)

Fig. 2. Diagram from a transverse section through the node of *E. maximum*, cutting two bifurcating bundles at slightly different relative levels. (× 10.)

Fig. 3. From a transverse section through a mature node of *E. maximum*, showing radial rows of cells intercalated between mature tissues of the bundle. (× 175.)

Fig. 4. Two radial rows, similar to those of Fig. 3, more highly magnified. (× 294.)

Fig. 5. From a transverse section through a mature internode of *E. maximum*; *cc*, carinal canal; *r*, remains of protoxylem; *g*, *g'*, side-groups of xylem differentiated during the elongation of the internode. (× 175.)

Fig. 6. From a transverse section through a node of a bud of *E. maximum*, showing fewer cells than Fig. 3 does; the intercalation of rows of cells (cambial activity) is just beginning. (× 175.)

Fig. 7. From a transverse section through the internode adjacent to the node from which Fig. 6 was drawn. This figure shows that, in the internode of a bud, the bundle attains about as many elements in radial thickness as does the mature internode (Fig. 5); whereas the adjacent node (Fig. 6) has not yet as many elements as the mature node (Fig. 3); thus there must be cambial activity. (× 175.)

Fig. 8. From a tangential section of a node of *E. maximum*, showing cambium-like cells in tangential view. (× 175.)

Fig. 9. From a radial section through a node of *E. maximum*, showing radial view of the cambium-like cells. (× 175.)

Fig. 10. From a transverse section of the internode of a young calamite showing a 'carinal canal' occupied by the remains of protoxylem; rings marked × lie in a lower focal plane. (× 294.)

Fig. 11. From same section of calamite-internode as Fig. 10, showing fascicular and inter-fascicular cambium and secondary tissue derived from it. (× 294.)

Fig. 1.

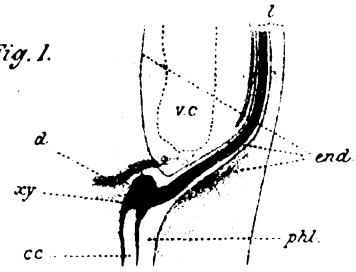


Fig. 2.

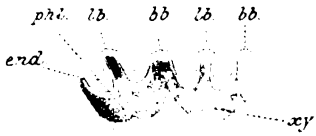


Fig. 4.

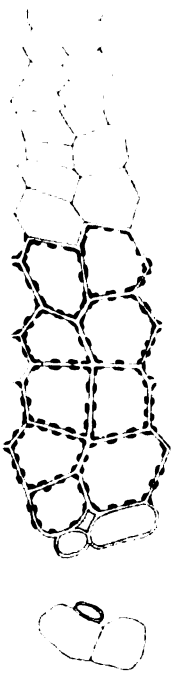


Fig. 3.

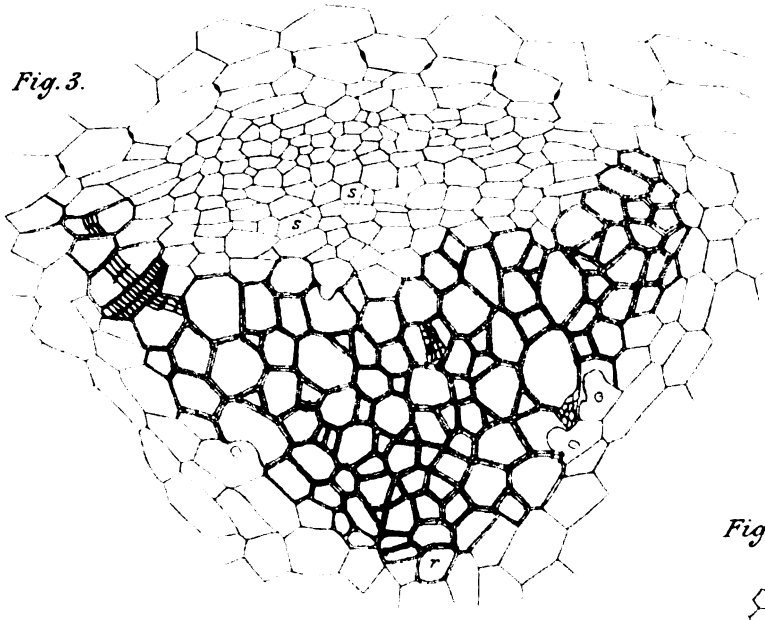


Fig. 6.

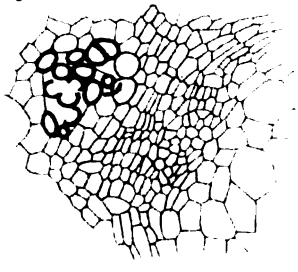


Fig. 5.

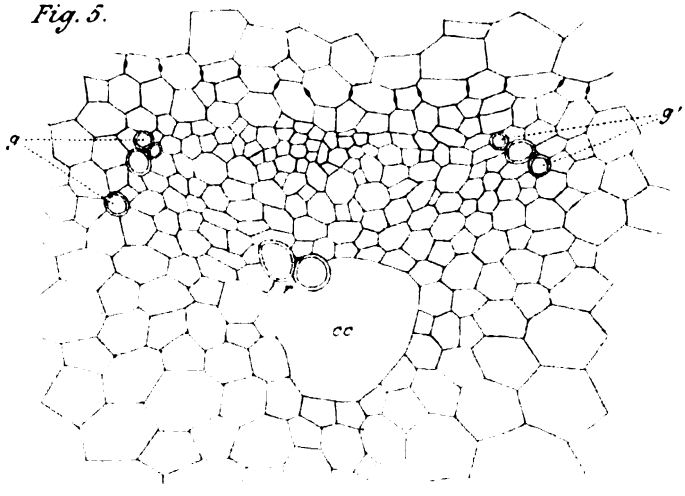
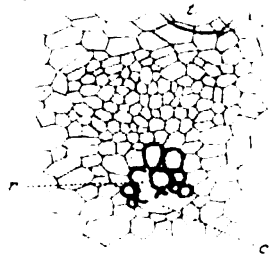


Fig. 7.



B. I. Cormack del.

Fig. 8.

Fig. 9.

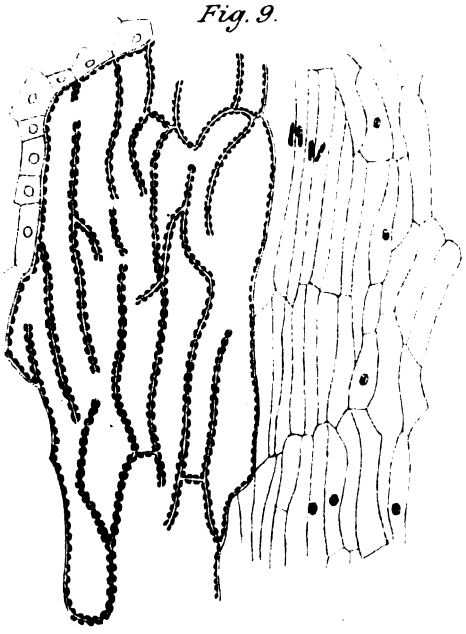


Fig. 10.

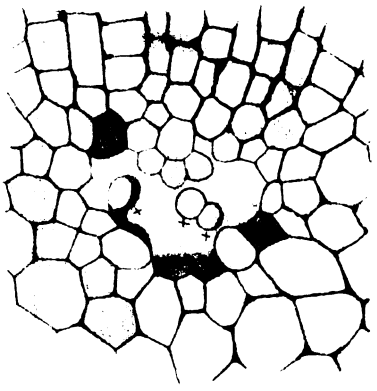
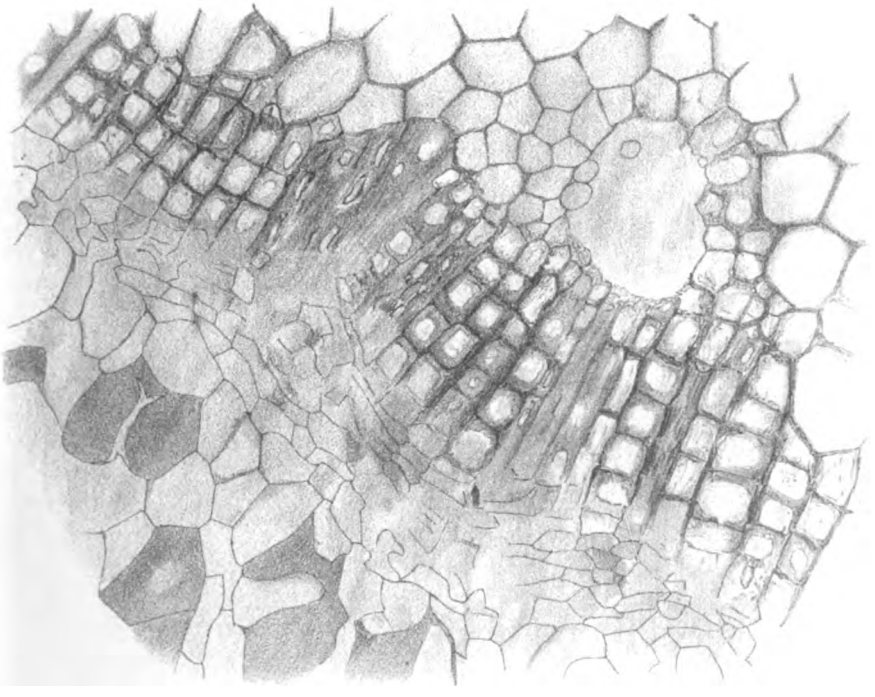


Fig. 11.



University Press, Oxford.

On Vegetable Ferments.

BY

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DURING recent years so many investigators have been occupied with the study of the several enzymes existing in various plants, and so many papers have appeared in different scientific journals, that it seems desirable to collect together the more important results that have been obtained and to present them in something like a consecutive form. This is the more needful, as the recent enormous development of bacteriology has led to the isolation of many enzymes from the so-called organised ferments, thereby opening to discussion the reality or necessity of the division hitherto held to exist between the latter and the enzymes themselves.

In the present paper the writer proposes to give some account of the various vegetable enzymes now known to exist; to review their general properties, mode of action, and composition, and to discuss briefly their relation to the other group.

Provisionally these bodies may be classified according to the materials on which they work. We may thus make four well-marked groups, excluding those which are obtainable from micro-organisms as well as one or two whose action has not been thoroughly investigated. These groups will be—

(1) Those which attack carbohydrates. These will include the different varieties of diastase, the ferment transforming inulin, the invertase which breaks up cane-sugar, the cytohydrolysts attacking cellulose, and the ferment which forms vegetable jelly from pectic substances.

[Annals of Botany, Vol. VII. No. XXV. March, 1893.]

(2) Those which decompose glucosides, with formation of sugar and various aromatic bodies. Of these the best known are emulsin or synaptase, myrosin, erythrozym, and rhamnase.

(3) The proteo-hydrolytic group, including vegetable pepsin, trypsin, and rennet, resembling very closely the animal enzymes bearing the same names.

(4) The enzyme that decomposes oils or fats.

Besides these well-marked groups, we have instances of the occurrence of others whose action is more special and confined to particular substances which do not seem to play a very important part in the metabolism of the vegetable organism in general. Such are the enzyme extracted by Lea from the cells of *Torula Ureae*, which decomposes urea with formation of ammonic carbonate, and that which according to Springer [1] occurs in the stem of *Nicotiana*, and has the property of decomposing nitrates and of forming butyric acid at the expense of sugar. Lastly, we have the various enzymes extracted from Bacteria.

These will be described separately and in order.

Carbohydrate-Enzymes.

Diastase. Recent observations made by several observers lead to the idea that there are two kinds of diastase existing in plants. The first of these has been shown by various writers to have a very wide distribution in plant cells. Baranetzky indeed suggests that it is universally present so long as the cells are living. Kjeldahl [2] found it in ungerminated barley, where recently it has been investigated by J. O'Sullivan, and by Brown and Morris [3] who find it also in the young embryo. Persoz and Payen [4], von Gorup Besanez [5], and others have found it in germinating seeds of various plants, Kossmann [6] and

[1] Nature, Oct. 16, 1884.

[2] Résumé du Compte rendu des travaux du Laboratoire de Carlsberg, 1889, I, p. 129.

[3] Journal of the Chem. Soc., June 1890, p. 505.

[4] Ann. de Chim. et de Phys. LIII, 1883.

[5] Sitzber. d. phys. med. Soc. zu Erlangen, 1874.

[6] Bull. de Soc. Chim. de Paris, XXVII, 1877.

Krauch[1] proved its existence in leaves and shoots, Baranetzky[2] in buds and in potato-tubers. Recently Wortmann[3] has denied its existence in leaves, attributing the conversion of starch into sugar to the direct influence of the protoplasm of the cells. Its existence there has however been reaffirmed by Vines[4] and others, who have criticised Wortmann's results. It has been found by the writer in the pollen-grains of several plants[5].

This body has been investigated in gradually maturing seeds of barley by Brown and Morris[6], who show that it makes its appearance in the developing grain at a very early period and gradually increases until the endosperm is fully developed, but the grain not ripened. Comparing the amount formed at three periods, when the endosperm is half-developed, when it has attained two-thirds of its development, and when it is complete, they find the relative quantities may be represented by the figures 4·4, 7·8, and 9·9. It is most plentiful always in the part of the endosperm nearest to the young embryo and appears to prepare the material for the nutrition of the latter as it is increasing in size. On germination, some time later, the diastase appears in the young embryo, both in the plumule and the radicle, though here the quantity is relatively small.

Its action may be examined upon the starch-grains *in situ*, or it can be extracted by water or glycerine, and its activity noted upon starch-paste, or on a preparation of soluble starch. In the former case Brown and Morris describe it as gradually dissolving the starch-grain from the outside only, without giving rise to any pitting or corrosion, the size of the grain gradually diminishing, while the shape and translucency are unaffected almost to the point of disappearance. When a weak starch-paste, containing about 1 per cent. of starch, is mixed with an extract of this form of diastase, it

[1] Landwirthsch. Versuchsstat. XXIII, 1879.
[2] Die stärkeumbildenden Fermente, 1878. [3] Bot. Zeitg., 1890.
[4] Brit. Assoc. Reports, Cardiff, 1891: also Annals of Botany, V.
[5] Ibid. [6] loc. cit.

acts very slowly, producing gradually a liquefaction of the paste and a subsequent conversion of the starch with sugar. When allowed to act upon a true solution of starch, the so-called soluble starch, the transformation into sugar is rapid. The solution of starch can be prepared by the method of Kjeldahl, by acting on starch-paste with a little malt-extract and boiling the mixture as soon as liquefaction is complete, or preferably by that of Lintner, preparing the solution by acting on the starch-paste with dilute hydrochloric acid and subsequently neutralising.

Another and more active form of diastase has been described by several observers, notably Brown and Morris[1] and Haberlandt, as being formed at the onset of germination in the seeds of several members of the Gramineae. To distinguish it from the former variety Brown and Morris have given it the name of 'diastase of secretion,' the first being called by them 'translocation-diastase.' They speak of it as originating, shortly after germination begins, in the epithelial cells covering the scutellum. The conversion into sugar of the grains of starch in the endosperm starts just under the scutellum and proceeds gradually towards the distal portion of the seed. The mode of dissolution of the starch-grains is essentially different from that caused by translocation-diastase. They become irregularly pitted, the fissures increase in number and depth, the outline of the grain becomes irregular and its laminae separate from each other, the grain becoming completely disintegrated before it disappears. The process is hence one of corrosion rather than of solution. When a solution of this form of diastase is mixed with starch-paste it rapidly liquifies it, converting it subsequently mainly into sugar. Embryoes removed from germinating barley-seeds and allowed to rest upon various preparations of starch-paste, and of gelatin containing starch-grains in suspension, were found to have a similar power of corroding and dissolving the granules, the latter undergoing the same changes as in the uninjured germinating seed.

[1] op. cit.

This form of diastase is absent from the resting seed and only makes its appearance at the onset of germination. That it is a true product of secretion is made probable by histological observations upon the epithelial cells of the scutellum in which its formation is asserted. The authors say that 'the histological changes in the columnar cells during germination are closely paralleled by those which occur in animal cells while actively secreting, and in the glandular cells of *Dionaea muscipula* under like circumstances, as determined by Gardiner.'

The existence of these two forms of diastase has been indicated also by Lintner and Eckhardt, in a comparison which they have made of the diastatic power of raw and germinated grain. They point out further differences between them as to certain features of their action, particularly with regard to the temperatures which are most favourable to their respective action. The optimum temperature for malt-diastase is between 50 and 55° C., while that for barley-diastase is at least 5 degrees lower. The diastatic power of the latter variety at 40° C. is as great as that of the former at 14·5° C.

Haberlandt[1] contends that the diastase of secretion has for its seat of formation also the so-called aleurone-layer of the barley-grain. He describes the cells of this layer as assuming in germination the general characteristic features of glandular cells, and projecting in papilla fashion into the interior of the endosperm. When this layer is isolated and grains of starch laid on it, Haberlandt says it corrodes and dissolves them. Brown and Morris dispute the accuracy of his experiments, attributing the results he obtained in such corrosion to the fact that the whole endosperm gradually becomes permeated with the ferment discharged by the scutellum, and that the cells of the aleurone-layer were consequently in contact with a solution of the diastase on their interior face.

Brown and Morris call attention to the fact that secretion

[1] Ber. der deut. botanischen Gesells., 8, 40.

cannot be induced in the epithelium-cells of the scutellum without previous expulsion of moisture.

The two varieties of diastase may be thus compared—

(1) Translocation-diastase.—Dissolves starch-grains without corrosion; has a very slow action on starch-paste, though it readily converts soluble starch into sugar; works best at a temperature of 45–50° C.; is much more energetic at a low temperature than secretion-diastase.

(2) Diastase of secretion.—Corrodes starch-grains and disintegrates them before solution; rapidly liquefies starch-paste; works most advantageously at a temperature of 50–55° C.

The action of diastase is one of hydrolysis. It is very rapid up to a certain definite point, when the mixture is found to consist of maltose and dextrin in the proportion of about four parts of the former to one part of the latter. The intermediate decomposition is of a very complex character. The most recent hypothesis is that advanced by Brown and Morris in 1889 [1]. They suggest that the starch-molecule has a formula of $5(C_{12}H_{20}O_{10})_{20}$ and is composed of five amylin or dextrin-like groups, four of the latter being arranged about the fifth. The first act of hydrolysis is the liberation of them from one another, four of them, by successive hydrolysations, being then converted through a series of amyloins or malto-dextrins to maltose, while the fifth withstands for a long time the action of the ferment. When the transformation of the four groups is complete, the condition noted above is arrived at, the proportion of maltose to dextrin being as 4 : 1.

The products which are formed in the plant by diastase have not been so completely examined. The final product of the starch is apparently maltose, but very little is known at present about the intermediate bodies by actual experiment.

The conditions under which malt-diastase works most

[1] On the analysis of a beer of the last century: Transactions of the Laboratory Club, No. 4, vol. III, February 1890.

advantageously have been recently investigated by Effront [1], who finds that its action is favoured by very small traces of mineral acids and by slightly larger amounts of common salt. In the presence of certain other bodies he finds its hydrolysing power is very greatly increased, particularly salts of phosphoric acid, certain compounds of aluminium, asparagin and some proteids. Experimenting with 1 cc. infusion of malt to 100 cc. starch-paste in the presence of these bodies he finds the following yield of glucose per 100 parts of starch:—

Malt-extract alone			8·63
„	„	with ·7 %	Hydric ammonic phosphate 51·63
„	„	„ ·5 „	Calcic phosphate 46·12
„	„	„ ·25 „	Ammonia-alum 56·3
„	„	„ ·25 „	Acetate of aluminium 62·4
„	„	„ ·02 „	Asparagin 37
„	„	„ ·05 „	Asparagin 61·2

Inulase. In various plants of the natural order Compositae, notably the Dahlia, the Artichoke (*Helianthus tuberosus*) and *Inula Helenium*, in different parts of their tissues, but especially in the tubers or tuberous roots, the ordinary carbohydrate reserve material takes the form of inulin and not of starch. Inulin has been considered to stand in the same relation to laevulose as starch does to dextrose. Starch is absent from the parts of the plant which contain inulin and no doubt the latter replaces it functionally. During the germination of the tubers of the artichoke the inulin is found to give place to sugar. During the slow maturation and the resting-condition of these tubers no ferment capable of bringing about this change can be extracted from them, but when germination begins, evidence of the existence of such a body is not lacking [2]. If the germinating tubers be minced or beaten up in a mortar and the pulp extracted with glycerine, the latter, when filtered till clear and mixed with a solution of inulin,

[1] Effront, Sur les conditions chimiques de l'action des diastases. Comptes rendus, cxv. p. 1324. Dec. 26, 1892.

[2] Green, Annals of Botany, vol. I, 1888.

gradually converts it into sugar. The stages of the decomposition appear to be as complex as those noted in the hydrolysis of starch, intermediate bodies with characteristic reactions being present in the mixture. This transformation has been found to be due to a special enzyme, to which the name *inulase* may be given. It is a different body from diastase, for it has no action upon starch-paste. Inulase is present in the tuber in very small amount, and probably only occurs at any moment in the cells in which the hydrolysis of the inulin is actually taking place. Unlike the diastase of secretion it gives no histological evidence of its formation.

Inulase is very sensitive to contact with acid or alkalis; not only is its activity impaired by the presence of more than a trace of either in the fluid in which it is working, but exposure to ·2 per cent. of HCl or 1·5 per cent. $Na_2 CO_3$ destroys it altogether. The destruction is more rapid at a moderately high temperature (40° C.) than at a lower one (10–15° C.). A trace of HCl, not more than ·005 per cent., is rather advantageous than not, but so slight is this acidity that it may be said to work best in a neutral medium. Its optimum temperature is 40° C., and like other enzymes it is destroyed by boiling.

Invertase. Another enzyme belonging to the first group is the body known as *invertase*, which is so named from its power of inverting cane-sugar, or hydrolysing it into dextrose and laevulose. Before cane-sugar can undergo alcoholic fermentation this preliminary change must be effected, and the yeast itself which brings about the former decomposition also causes the hydrolysis, a fact ascertained by Dubrunfaut in 1847. Hansen[1] has shown that invertase is present in several other micro-organisms; Brown and Heron[2] found it to be present in the cold-water extract of malt; Kossmann[3] detected it in the buds and leaves of young trees, and Van Tieghem[4] in the pollen-grains of certain plants. Bechamp[5]

[1] Medelelser, 1888, 2. 143.
[2] Trans. Chem. Soc. 35, 1879, 609.
[3] Comptes rendus, 81, 406.
[4] Bull. Soc. Bot. de France, t. 33, 1886.
[5] Mém. Acad. Sci. 28, 347.

found a similar body in the petals of *Robinia pseudacacia* associated with another enzyme having diastatic powers. It has been shown by Kjeldahl[1] and by J. O'Sullivan[2] to be present in the embryo of germinated barley, particularly in the rootlets, from which however it is by no means easy to extract it. J. O'Sullivan finds it also in the plumule. Sachs suggests its existence also in the wintering beet-root and the fruiting spikes of *Zea Mais*. Wassezug[3] has found it in certain fungi of the genus *Fusarum*, which have the power of growing in cane-sugar solutions, causing in them formation of glucose. When the fungus is cultivated in bouillon made from veal, he says it excretes a little of the enzyme into the liquid at the period when it forms its conidia. Fernbach has extracted it from *Aspergillus niger*[4].

It is not confined to the vegetable kingdom, occurring also in parts of the mammalian alimentary canal.

Various methods for its preparation have been given by different authors. Berthelot obtained it in solution in 1860, and Hoppe-Seyler[5] prepared it from yeast in the form of a soluble powder in 1871. He killed the yeast with ether, extracted it with water and precipitated the invertase by alcohol. Other authors have modified the process in their experiments, but their methods are based upon Hoppe-Seyler's. Gunning[6] extracted it from washed yeast by means of glycerine. O'Sullivan and Tompson[7] obtained it in quantity by pressing good sound yeast for several weeks till it liquefied, and then filtering off the liquor from the residue. This filtrate contained all the invertase of the yeast, amounting to from 2 to 6 per cent. of the dry solid matter of the latter. From this filtrate they separated the enzyme by adding alcohol to 47 per cent., when it was precipitated. To purify it they washed it with spirit of the same strength, again ex-

[1] Résumé du Compte rendu des travaux du Laboratoire de Carlsberg, 1881.
[2] Transactions of the Laboratory Club, No. 5, vol. III.
[3] Ann. de l'Institut Pasteur, 1, p. 525, 1887.
[4] Ibid, 1889, p. 473.
[5] N. Rep. Pharm. 20, 764.
[6] Ber. 5, 821.
[7] Journ. Chem. Soc., Oct. 1890, p. 834.

tracted it with alcohol of 10–20 per cent strength and filtered, when the filtrate was found to contain all the enzyme in a very pure condition. It can also be prepared as a dry powder by dehydrating the first precipitate and drying it in vacuo.

The action of invertase on cane-sugar may be expressed by the equation:—

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Laevulose.}}{C_6H_{12}O_6}$$

It is a very unstable body and is easily damaged or destroyed, the caustic alkalis even in very small proportions being especially destructive. It resembles inulase in that a very minute trace of mineral acid favours its activity, but a very slight additional quantity is detrimental. The most favourable amount of acidity, sulphuric acid being used[1], varies with the amount of invertase present, and with the temperature of digestion. The more of the enzyme that is in the solution, the greater is the amount of acid required for the maximum effect. Thus with ·4 per cent. of invertase present, the optimum amount of sulphuric acid is 12·5 parts per million of the solution; with 1·5 per cent. the amount rises to 15 per million, the temperature being 56° C. If the experiment he conducted at 15·5° C., when 1·5 per cent. of invertase is used, the acid required is 75 parts per million, while if 15 per cent. of the ferment is present, the acid must be 250 parts per million.

The influence of temperature under these conditions also appears from a comparison of these figures. Taking the invertase present as 1·5 per cent., the amount of acid required for the best results at 56° C. is 15 per million of solution, but at 15·5° C. it is 75 or 5 times as much. Excess of acid, even of very little, is prejudicial. Thus at 60° C. an excess of only 2 parts of acid per million lowers the rapidity of the action elevenfold.

Alcohol exerts on the whole a deleterious influence, varying in proportion to the amount present. With 5 per

[1] O'Sullivan and Tompson, op. cit.

cent., the speed of the hydrolysis is reduced by one half. The ferment is precipitated uninjured by 47 per cent. of alcohol, but a larger proportion decomposes it, as when the resulting precipitate is redissolved it is found to be inert towards sucrose.

The optimum temperature for the action of invertase is between 55 and 60° C. At 65° C. it is gradually and at 75° C. rapidly destroyed. Below the optimum temperature the activity gradually diminishes.

Invertase works best in a cane-sugar solution of the concentration of about 20 per cent., the activity being slightly lessened as the sucrose is increased in amount to 40 per cent., while in saturated solutions inversion proceeds very slowly. The products of the inversion seem to have no inhibitory influence on the working of the enzyme, a point in which it forms an exception to the general rule. It is not exhausted by its activity.

Fernbach [1] noted that his extract from *Aspergillus* was less active in light than in darkness, and that the inhibitory effect was the greater as the extract was made gradually more acid.

Cyto-hydrolytic Enzymes. In the endosperms of the Palms the carbohydrate reserve materials take the form of cellulose, the walls of the cells being so enormously thickened that their cavities seem to be almost obliterated. As these walls gradually disappear during germination it seems probable *a priori* that the seeds contain an enzyme for its transformation into some soluble product. Many observers have endeavoured to detect the presence of such a body, either in the embryoes or the endosperms of various species, but hitherto without success. Most experiments have been conducted on the seed of the date (*Phoenix dactylifera*). The well-known figure in Sachs' text-book accurately represents the various stages in the growth of the embryo, part of the cotyledon of which is transformed into an absorbing organ, or haustorium, which gradually softens, corrodes, and dissolves the hard cellulose of the endosperm. A section of this haustorium shows it to be

[1] loc cit.

covered with an epithelium, the appearance of whose cells certainly suggests a secretory activity resembling that of the scutellum of the barley. The outer walls of the cells composing this epithelium are thicker than those of the similar membrane of the latter, and it is difficult to see how the protoplasm contained in them can exert any direct action upon the endosperm. This, together with the granular appearance of the contents of the cells during the period of absorption, certainly points to a secretory activity leading to the excretion of an enzyme into the endosperm. The walls of the latter become softened so as to be easily cut and are then irregularly corroded and broken down. In another Palm (*Livistonia*) this disappearance of the cellulose is associated with the appearance of sugar, which can be demonstrated in the disintegrating endosperm and in the absorbing cells of the haustorium. A little deeper in the tissue of the latter, starch-grains make their appearance long before any leaf has been developed in the young embryo. The endosperm-cells, when extracted with the usual solvents, fail to yield any evidence of the presence of an enzyme, nor is there any satisfactory proof of the existence of such a body in the epithelium of the haustorium, though certain experiments carried out by the writer[1] appeared to show that there was a trace of one present. These results have not however been confirmed by subsequent observers, and up to the present therefore the search for a cytohydrolyst in the Palms has not been successful, though probably only better methods of experiment are required to establish its existence.

We have, however, evidence that the vegetable kingdom contains such bodies, and that in probably not exceptional cases.

De Bary in 1886 published particulars of some experiments on the Pezizas of the Sclerotinia-group in which he noticed a behaviour of the hyphae which suggested to him the occurrence of an enzyme there. When he cultivated these fungi on the pulp of carrots and turnips, he found that

[1] Phil. Trans. vol. 178 B. p. 57, 1887.

the tissues became softened, the mycelium destroying the cell-walls of the pith and cortex. The hyphae could be seen growing between the cells and breaking down the middle lamella. When the affected pulp of the carrot was pressed so as to obtain the juice from it, the latter was found to possess the property of dissolving cellulose. Pieces of vegetable tissue placed in such expressed juice were almost disintegrated in a few hours, the cell-walls swelling and the middle lamella being dissolved. The fluid expressed from the sclerotia of the fungus was still more effective. De Bary concluded the power of action was vested in an enzyme, as the juice lost its property on being boiled.

In 1889 Marshall Ward, while pursuing some investigations into the life-history of a *Botrytis* which was causing a particular disease in the Lily, seems to have met with the same ferment [1]. The *Botrytis* was found to be capable of penetrating the cell-walls of *Lilium candidum* and of growing freely inside the tissues. It is therefore parasitic as well as saprophytic in its habit. Certain of the hyphae, on coming into contact with any object such as a cell-wall, or a cover-glass, swell at the tip and apparently pour out a somewhat glairy fluid; the hyphae branching at the same time below the point of contact. If such a hypha does not come into contact with anything, it gradually pours out a nearly transparent viscid drop of fluid, the exudation lasting for some hours. The drop gradually becomes very granular, with brilliant refringent granules, and is found to give proteid reactions. From large cultures of the fungus made in Pasteur's solution a mass of such hyphae can be obtained, and from such a mass a watery extract containing the dissolved granular matter can be prepared. When thin sections of parenchyma are placed in this and kept warm, the cellulose is found to swell, show lamination, and ultimately undergo solution. If the watery extract be poured into a large excess of alcohol, a precipitate, partly crystalline and partly amorphous, rapidly forms. This can be separated by filtration,

[1] Annals of Botany, II. 2, 319, 1888.

washed and dried, when it forms a greyish powder. This powder is largely soluble in water and is partly composed of the ferment. When an extract of it is allowed to act upon parenchyma-cells the results above described are soon to be seen, the ferment dissolving the middle lamella and gelatinizing the cell-walls. None of these extracts produced any change in the parenchyma if they were boiled before the experiment.

Marshall Ward is inclined to attribute the branching seen in these hyphal filaments just below their attachment, to the presence of the ferment. He thinks that it is caused by a local action or accumulation of the enzyme softening the wall of the hypha just below the apex, and the pressure within then causing protrusion and so growth. Indeed he thinks it possible that the apical growth of the hypha may be attributed to a similar condition.

A similar cellulose-dissolving enzyme, or cytohydrolyst, has been discovered by Brown and Morris in the germinating barley-grain [1]. During germination the cells underlying the scutellum undergo a softening and partial dissolution of their walls, becoming isolated from each other before their starchy contents are attacked by the diastase. The cell-walls swell and the several lamellae partially separate, showing marked stratification. The lamellae gradually dissolve, the middle lamella being the most resistant. Ultimately the wall undergoes fragmentation and disappears. It is not till after a certain amount of this action has taken place that the starch-grains in the cells are attacked, but in the meantime newly formed starch-grains make their appearance in the cells of the scutellum part of whose function is absorptive. The course of events appears to be the transformation of the cellulose into some carbohydrate capable of dialysis, probably some form of sugar, the absorption of this by the scutellum, and the formation of the transitory starch at its expense.

The granular character of the protoplasm of the cells of the epithelium of the scutellum has already been referred to

[1] op. cit.

in connection with the diastase formed there. The action of the epithelium on the walls as well as on the contents of the underlying cells suggests that it secretes a cytohydrolytic ferment as well as a diastatic one.

To demonstrate the existence of this enzyme is not difficult. If a cold-water extract of air-dried malt be precipitated by excess of alcohol, the precipitate collected, dehydrated, and dried in vacuo, it forms a white powder which is soluble in water. If a solution of this be prepared and a section of a barley-endosperm be placed in it, the dissolution of the cell-walls proceeds just as in a germinating seed. Boiling the extract renders it inert.

This extract will dissolve the cellulose of plants other than the Barley, though it is not a universal solvent for this material. It has been found to have no action on the cellulose of the endosperm of the Date, nor on the parenchyma of the Apple. It acts but slowly on the thickened cell-walls of the endosperm of *Bromus mollis*.

Like other enzymes, it seems to work best in a very faintly acid medium, formic or acetic acid being most favourable.

Though produced in the same cells as the diastase of secretion the authors consider it to be a separate ferment. Its action precedes the action of the diastase and the temperature at which it is destroyed is lower. It becomes much injured by exposure to 50° C., and almost paralysed if heated for half an hour to 60° C. The diastase survives heating to 70° C., not being perceptibly injured at that temperature. The details of its action are not yet known; probably it forms some kind of sugar.

The histological changes noticeable in the cells of the scutellum throw some light on the probable mode of secretion in the vegetable organism. This point will however be treated in more detail after examining other cases.

A difference between the cytohydrolytic and the other enzymes treated of so far, may be noted here. Brown and Morris call attention to the fact that it is only formed as the food-materials in the secreting cells diminish, and that an in-

crease of them inhibits the secretion. Marshall Ward noted a similar fact in his experiments with *Botrytis*.

Pectase. One of the earliest known ferments of the vegetable organism was described by Fremy in 1849[1]. He says that the cell-wall is largely composed of a substance to which he gives the name of pectose, which differs from cellulose in many of its reactions. Pectose, or pectin, by the action of an enzyme existing in certain cells, can be converted into two gelatinous bodies, pectosic and pectic acids. The transformation proceeds by two stages, the two acids being formed successively. They differ from pectin chiefly in the amount of water they contain. Pectase, as Fremy calls the ferment, exists in two conditions in the vegetable organism; from the carrot and the beet it can be extracted by mashing the roots and expressing the juice from the pulp; in acid fruits it exists in an insoluble condition. If juices of the pulp of these be put into a solution of pectin, they cause a very rapid gelatinisation, forming as before pectosic and later pectic acid. From the juice of young carrots pectase can be precipitated by alcohol. Its optimum working temperature is 30° C., and it is destroyed by prolonged boiling. It can work in the absence of oxygen.

A ferment of this kind is described by Wiesner[2] as obtainable from gum-arabic. He speaks of it as transforming cellulose into gummy or mucilaginous substances. Reinitzer[3] denies the cellulose-transforming power, and says that the ferments extractable from gum are diastatic.

Glucoside-Enzymes.

The next group of ferments that we shall consider are somewhat like the foregoing in that they aid in furnishing the plant with a supply of soluble diffusible carbohydrate material in the form of sugar. They differ however in their

[1] Ann. Chim. et Phys., Sér. 3, vol. XXIV, p. 1.

[2] Wiesner, Sitzungsb. d. k. Akad. d. Wissensch. in Wien, xcii, p. 140: also Bot. Zeitg., 1885.

[3] Reinitzer, Ueber die wahre Natur des Gummifermentes, Zeitschr. f. Phys. Chem. 1890.

action in that they decompose certain complex bodies known as glucosides, splitting off sugar from their molecules, but giving rise also to a variety of other bodies, many of which, so far as we know, are of no use in the nutrition of the plant. The process which they set up is in most cases, like the former, one of hydrolysis, the entry of water and the disruption of the molecule of the glucoside taking place simultaneously. From the greater complexity of the decomposition, Sachs is inclined to look upon the two classes of ferments as radically different from each other, but probably this is not the case, the complexity arising from the nature of the body hydrolysed, the ferment in both cases being responsible only for the actual hydrolysis. Thus the action of emulsin, one of these bodies, on amygdalin is expressed by the equation:—

$$\underset{\text{Amygdalin}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\text{Benzoic aldehyde}}{C_6H_5COH} + \underset{\text{Prussic acid}}{HCN} + 2\underset{\text{Sugar.}}{(C_6H_{12}O_6)}$$

The best known members of this group are the *emulsin* of the bitter almond, the *myrosin* of the black mustard and other Cruciferae, the *erythrozym* of the madder-root, and the ferment found by Marshall Ward and Dunlop in the seed of *Rhamnus infectorius*.

Emulsin has sometimes been called *synaptase*. It has long been known to be present in certain species of *Amygdalus* and *Cerasus* or *Prunus*, from which it can be extracted in the form of a greyish powder. Its presence is associated with the formation of prussic acid, especially in the bitter almond and the cherry-laurel. It decomposes the glucoside amygdalin according to the equation just quoted. For a long time its distribution in the plant was uncertain, though it could be detected in all the parts where metabolism was vigorous. In 1865 Thomé[1] made some experiments upon the sweet and bitter almond, which led him to form the opinion that the enzyme existed only in the bitter variety and was localised there in the fibro-vascular bundles of the cotyledons. Portes[2] in 1877 came to the conclusion that emulsin was confined to the axis

[1] Bot. Zeit. 1865, p. 240.

[2] Portes, Journ. de Pharm. et de Chimie, t. XXVI, p. 410, 1877.

of the embryo, and that amygdalin was only present in the cotyledons. In 1877 Pfeffer[1] gave as his opinion that both ferment and glucoside were present together in the cells, the former being in the protoplasm, the latter in the cell-sap. In 1887 Johansen[2] found that emulsin exists in both varieties of the almond, and is distributed in the fibro-vascular bundles and the cells abutting on them, particularly in those of the cotyledons. He found amygdalin in the parenchyma of the cotyledons of the bitter variety only. In 1890 Guignard[3] published the results of a very careful research into the distribution of the ferment in both the almond and the cherry laurel. His work was partly based on micro-chemical methods, while he confirmed his results by observing what parts of the tissues had, when isolated, the power of liberating HCN from a solution of amygdalin. In his work he quotes two micro-chemical reactions on which he found himself able to rely. One of these is the development of a violet colour in cells containing emulsin when a section is treated with a solution of orcin in hydrochloric acid. The second is the behaviour of the same cells with Millon's reagent. Instead of the pale brick-red or rose-red which proteids give with this fluid, emulsin gives a much deeper and more persistent orange-red colouration. Certain layers of tissue which gave these colour-reactions were found, when very carefully isolated by dissection, to be capable of liberating HCN from a weak solution of amygdalin, and yielding at the same time the characteristic odour of benzoic aldehyde.

Guignard found the distribution of the enzyme in both the cherry-laurel and the almond to be in the neighbourhood of the fibro-vascular bundles, but not in quite the same layer in the two cases. In the case of the first named plant he prepared it from leaves and twigs, and located it chiefly in the endodermis. In the almond he only detected it in the seed and young seedling, where it was chiefly in the pericycle of the

[1] Pflanzen-Phys., t. I, p. 307, 1881.
[2] Ann. des Sc. Nat. Bot. sér. 7. t. VI, p. 118, 1887.
[3] Journal de Botanique, 1890, p. 3, et seq.

fibro-vascular bundles of the axis and of the cotyledons. In the axis he thought it extended also, though only to a small extent, to the procambial tissues; in the fibro-vascular bundles of the cotyledons it extended to the endodermis, but the latter contained very little.

Emulsin decomposes not only amygdalin, but many other glucosides, including salicin and coniferin.

Myrosin is the characteristic enzyme of the Cruciferae, though probably it is not confined to the plants of this natural order. Cruciferous plants abound in very complex glucosides, which on decomposition break up into sugar and various strongly-smelling compounds usually containing sulphur. One of the most commonly occurring ones is *sinigrine* or myronate of potassium, whose decomposition can be represented by the following equation:—

$$\underset{\text{Sinigrine}}{C_{10}H_{18}NKS_2O_{10}} = \underset{\text{Sulphocyanate of Allyl}}{C_3H_5CNS} + \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Potassic hydrogen sulphate.}}{KHSO_4}$$

When the seed of the black mustard (*Sinapis* or *Brassica nigra*) is bruised and treated with water the odour of the sulphocyanate of allyl is easily recognisable. Both the myrosin and the glucoside are contained in the seed, and the reaction is the result of their being brought together by the solvent.

The localisation of myrosin has been the object of a very elaborate research by Guignard [1], who has investigated a very large number of the genera and species of the Cruciferae. In 1886 Heinricher [2] showed that in many of the plants of this natural order special cells, very variously distributed, could be recognised by the peculiar nature of their contents. As these gave very strongly-marked proteid reactions, he considered them to be reservoirs of albuminoid material. By similar tests to those he employed in the case of the cherry-laurel and almond, Guignard identifies these as the cells that contain the enzyme. They are recognisable by their finely granular contents and by their being without starch, chlorophyll, fatty

[1] Journal de Botanique, Nov. 1890, p. 385, et seq.
[2] Mittheil. aus dem Bot. Inst. zu Graz, 1886.

matter, and aleurone-grains, though situated in various regions among cells containing one or more of these constituents. They contain, associated with their protoplasm, a quantity of amorphous proteid matter which is coagulated by alcohol and then separates from the peripheral protoplasm in the form of coarse granulated masses, which are coloured by Millon's reagent a more vivid red than is the protoplasm. They can be distinguished among the parenchyma-cells in which they lie by staining with methyl-green and other anilin-dyes. Usually they are very slightly larger than the surrounding cells, being longer and less regular in shape.

Guignard finds these special cells distributed in all the parts of the plant, those found in the seed being the richest in ferment. In the root they exist in the parenchyma of the cortex and of the bast; in some cases also in that of the wood; in the stem they may be found generally everywhere, but especially in the pericycle; in the leaves they are disposed in the same way as in the stems which bear them; in the carpels much as in the leaves; in the ovule especially in the external integument. In the developing embryo these cells may be first detected at the time when its tissues begin to receive their deposits of reserve materials.

As in the case of the almond, the glucoside is deposited in different cells from those which contain the enzyme.

Guignard demonstrated the presence of the ferment in these cells most easily in the Wall-flower, where they form a readily separable layer in the pericycle. Isolating this with great care, and warming it with a weak solution (2 per cent.) of the glucoside, the characteristic odour of sulphocyanate of allyl was at once perceptible. He found throughout his experiments that any tissue containing these cells could effect the decomposition, but that if these were not present, the tissue could not act upon the glucoside.

Myrosin appeas to be capable of acting on all the glucosides which the various cruciferous plants contain, yielding characteristic results in each case.

The optimum temperature for its activity is a little below

50° C.; above that point it is less powerful and is destroyed at about 70° C.

The action of myrosin is peculiar, as the intervention of water is not necessary for the decomposition which it sets up.

Rhamnase. A third ferment belonging to this group has a more limited distribution than either of the two already described. It occurs in the seeds of *Rhamnus infectorius*, the so-called 'Persian berry,' a species whose fruits yield a yellow dye. This ferment, which may be called *rhamnase*, has been investigated by Marshall Ward and Dunlop[1]. The fruits contain a glucoside *xanthorhamnin*, to which the formula $C_{48}H_{66}O_{29}$ has been ascribed. When decomposed, it yields *rhamnetin* or *rhamnin* and glucose. If the pulp of the fruits, or an extract of the pericarp, is treated with an extract of the seeds and kept at 35° C. for a short time, a copious yellow precipitate falls, which consists of the rhamnin, the sugar remaining in solution. Boiling the extract of the seeds destroys its power of producing the precipitate. Very careful histological investigations proved that rhamnase is confined to the raphe of the seed, which is composed of parenchymatous cells, containing a brilliant oily-looking, colourless substance. The cells contain two or three large vacuoles, in which a few brilliant granules can be observed. The glucoside, as in the other cases, does not exist in the same cells as the enzyme, but is confined to the pericarp and pulp of the fruit, in which it is very abundant. The rhamnase can be extracted from the raphe, either by water or glycerine.

Besides these glucoside-splitting ferments several others are known to occur, but they have not been so completely examined. The **Erythrozym** of madder-root has already been mentioned. Others are referred to by Schützenberger[2] as being found in various plants. One of them is capable of decomposing phillyrin, a glucoside present in the bark of *Phillyrea latifolia*, and populin, from the bark of the Aspen. Another splits up tannin into gallic and elagic acids and sugar.

[1] Annals of Botany, vol. I, 1887.

[2] On Fermentation. Internat. Scientific Series, vol. XX.

It has quite recently been claimed by Sigmund[1] that these enzymes have also the power of splitting up fats into glycerine and free fatty acids. He says that he caused myrosin and emulsin to act upon olive-oil in closed glass vessels at a temperature of 38° to 40° C., and that gradually and continuously free fatty acid was developed in the mixture, its presence being demonstrated both by litmus and phenol-phthalein.

His mode of preparing the enzymes is, however, open to criticism. He bruised seeds of the mustard in one case, and of the almond in the other, with excess of water, and allowed them to extract for twelve or fourteen hours. He then decanted the supernatant fluid and added excess of alcohol, throwing down a precipitate which he removed by filtration, washed and dried at about 40° C. This method is hardly likely to prepare either myrosin or emulsin pure; if any other ferment, e. g. a fat-splitting one, were present in the seeds as well as the former, it would certainly be present in his dried residue. Though hitherto no one has attempted to isolate a fat-splitting enzyme from these seeds, there seems to be ground for suspecting its presence, as both mustard-seeds and almonds contain oil. Sigmund further states[2] that certain fat-splitting enzymes which he detected in various seeds, as will be mentioned in connection with other researches on this point[3], were able to split up amygdalin and salicin. The same criticism may be applied to this statement. The mode of extraction was similar and it is at least possible that his residue contained two ferments, rather than one as he supposes.

Proteo-hydrolytic Enzymes.

The ferments or enzymes which effect the decomposition of proteids, and to which therefore the name *proteo-hydrolytic* may be applied, have been the subjects of observation and experiment by many writers since 1875. The

[1] Sigmund, Beziehungen zwischen fettspaltenden und glycosidspaltenden Fermenten. Sitzungsberichte d. k. Akad. der Wissenschaften in Wien, Math.-Nat. Classe, Bd. 101, May 1892.

[2] loc. cit.

[3] Vide infra, p. 116.

first work which calls for notice is that which was carried out by Reess and Will[1] of Erlangen on the leaves of *Drosera rotundifolia*. Working on the same lines as animal physiologists, they treated the leaves with strong alcohol to dehydrate them and then, after reducing them to pulp, they extracted them with glycerine. This extract, made acid with dilute hydrochloric acid, was found capable of dissolving swollen-up fibrin at a temperature of 40° C., and the resulting liquid gave the reactions of peptone. Careful control-experiments proved that the power was due to the presence of a soluble enzyme. In 1876 von Gorup-Besanez[2] demonstrated the existence of a similar body in the pitchers of *Nepenthes*, and his results were confirmed and extended by Vines[3] a little later. Since that date proteohydrolytic ferments have been discovered in several plants and have been more critically examined.

Writers on animal physiology have generally classified these enzymes into two groups; the first, of which the pepsin of the stomach is representative, being capable of converting proteids into peptones, probably by hydrolysis; the other, illustrated by the trypsin of the pancreas, carrying the digestion further, and decomposing some of the peptone into nitrogenous crystalline bodies, chiefly amides such as leucin and tyrosin. Both of these groups appear to have representatives in the vegetable kingdom.

Pepsin. The members of the *peptic* group were the earliest known. Such are the ferments of *Drosera*, *Dionaea*, *Pinguicula*, and the other insectivorous plants, with probably those of the pitcher-plants *Nepenthes*, *Sarracenia*, &c. Our knowledge of the former group is due in greatest measure to the labours of Darwin[4].

The ferments in all these are secreted by the leaves, which are furnished with glandular structures capable, on being stimulated, of pouring out a peculiar secretion which possesses peptic powers. When an insect, or a small piece of nitro-

[1] Bot. Zeit., Oct. 29, 1875, No. 44.
[2] Berichte d. deutsch. chem. Gesellsch. zu Berlin, May 22, 1876.
[3] Journ. Linn. Soc. Bot., vol. XV, p. 427.
[4] Insectivorous Plants.

genous matter, is placed upon a leaf of *Drosera*, the glands quickly exude a somewhat viscid slightly acid fluid, at the same time bending over to imprison the stimulating matter. The surface and margins of the leaves are alike provided with stalked glands, the secretion of the central ones being more acid than that of those at the periphery. Darwin considered the acid to be propionic, or else a mixture of acetic and butyric acids. The ferment of the secretion acting in the acid medium dissolves the nitrogenous body imprisoned by the glands. Besides proteid matters, it can dissolve connective tissue, cartilage, and gelatin; but it has no action on mucin. Besides resembling animal pepsin in the medium in which it works and in the decompositions it effects as just described, it is much like it in the conditions of its secretion, being found in the exuded fluid only when the glands have been stimulated by the absorption of nitrogenous matter. The acid of the juice of *Drosera* is only developed under the same conditions.

Darwin discovered that the same ferment exists also in the leaves of *Dionaea*. These differ in the arrangement of their glands from those of *Drosera*; the leaves have their upper surfaces covered with small almost sessile secreting glands of a purplish colour. Like the leaves of *Drosera*, those of *Dionaea* do not secrete anything until they are excited by the absorption of nitrogenous matter. Then they pour out a fluid which is colourless and slightly mucilaginous. It is more acid than that of *Drosera*, and acts like the latter on albumin. *Pinguicula* also secretes a similar body on the edges of the upper surface of the leaf which folds over to enclose its captives.

To von Gorup-Besanez[1] and to Vines[2] we are indebted for our knowledge of the powers of the liquid in the pitchers of *Nepenthes*. The former established the fact that the fluid could dissolve fibrin when the latter was placed in it and kept for an hour at a temperature of 40° C., the solution giving the biuret reaction characteristic of peptone. The exact

[1] op. cit. [2] op. cit.

nature of the action was however not investigated, and we can say nothing more as to the various bodies formed in the digestion. Vines carried the work further by showing that the enzyme can be extracted from the walls of the pitcher by dehydration and subsequent treatment with glycerine. The action can only be detected when the ferment works in a faintly acid medium, about ·2 per cent. of hydrochloric acid being best. Vines' paper embodies further some experiments made upon the pitchers with the view of ascertaining in what condition the ferment exists in the pitchers when no digestion is provoked. To this point we shall return later. Krukenberg[1] obtained a similar ferment from the plasmodium of *Aethalium septicum*, one of the Myxomycetes. Besides peptone, he found a body resembling an albumose in the products of the digestion.

These ferments appear to resemble very closely the pepsin of the stomach, and provisionally they must be classed with it. In the characters of their action, their intimate association with weak hydrochloric or other acid, and the materials they can dissolve, the resemblances are striking. At the same time it must be remembered that the various authors do not quote any very detailed account of the bodies formed during the action, leaving it therefore undetermined whether the power of the ferment is sufficient to split up peptone into amide-bodies.

Trypsin. Of the tryptic group the ferment which was the first to be very completely examined is the so-called papaïn or papaïne. It has long been the custom of the natives of India to cook certain fruits with tough meat to make it tender, and curious stories have obtained currency with respect to the powers of that of the Papau (*Carica Papaya*) in this connection. Wrapping tough meat in the leaves of the plant, or even hanging it under the tree has been said to have the same effect. Three such fruits in particular have been used for the purpose, the Papau, the Fig, and a variety of the Melon (*Cucumis utilissimus*).

[1] Untersuch. aus dem physiol. Inst. der Univ. Heidelberg, Band II, Hft. 3, 1878.

Underlying the curious stories current among the natives as to the powers of these fruits, there has been ascertained to exist in each of them a proteo-hydrolytic ferment. The Papau was the first to be investigated, and to the labours of Wurtz[1] and of Sidney Martin[2] we are indebted for a fairly complete knowledge of its properties and powers. Wurtz found that in the juice of the stem, leaves, and fruits of this plant, an enzyme exists which digests various kinds of proteids. It can be prepared by expressing the juice, precipitating therefrom the enzyme in a very impure condition by strong alcohol, dehydrating the precipitate and extracting it with water. Wurtz considered it to be a proteid body; the extract containing it was neutral in reaction and became cloudy on boiling. Probably his body was by no means pure, judging from the method adopted to prepare it. It acted rapidly in a neutral medium, dissolving animal proteids, and forming chiefly peptones, but also crystals of leucin.

A much more complete examination of it was made in 1883 and 1884 by Martin. He prepared it from the commercial papaïne obtainable in the market, and found it to be intimately associated with proteid matter existing in the fruit or latex. In his experiments he used both animal and vegetable proteids, and found it capable of digesting both.

Working on fibrin and on egg-albumin, Martin says that the action is one of corrosion of the proteid matter, rather than one of solution. In the experiments the fibrin was gradually converted into a pultaceous mass, a good deal of turbidity accompanying the action, just as in the case of digestion of similar material by the trypsin of pancreatic juice. The optimum temperature was 35° to 40° C., but at lower temperatures such as 15° C., it was also active. He agrees with Wurtz in finding that it will work in a neutral medium, but says that the activity is much greater when a little Na_2CO_3 (about ·25 per cent.) is added. Greater alkalinity than this is prejudicial, while very slight acidity, even ·05 per cent. HCl.,

[1] Comptes Rendus, 1879, p. 225, 1880, p. 1379.
[2] Journal of Physiology, vol. V, 1884.

is inhibitory. The products of the digestion under the most favourable conditions are an albumose, peptone, and both leucin and tyrosin. No alkali-albumin is found as is the case with pancreatic trypsin. Similar results were obtained with the vegetable proteids existing in the papau-fruit.

This ferment was the first one known of the second group, which may be called the vegetable trypsins.

The Fig, the second of these Indian fruits, has been examined by Bouchut [1] and by Hansen [2], who published his results in 1884 and 1885. He discovered in it a proteohydrolyst working best in an acid, but also, though less readily, in an alkaline medium.

The third, the *Cucumis utilissimus*, Roxb., was investigated last year by the writer [3], from a fruit which was grown at Kew from seed sent over from India by Brigade-Surgeon Bonavia. The ferment is found in the juice and pericarp, and is associated there with a globulin-like proteid. It is most effective in an alkaline medium, less so in a neutral one, and least of all in the presence of acid. Like papaïn, it effects a very complete decomposition of the proteid, giving rise to peptone and later to leucin.

Besides these Indian plants, a proteohydrolytic enzyme has been ascertained to exist in the juice of the pine-apple (*Ananassa sativa*), attention being first called to it by Marcano of Venezuela [4] in 1891. The fruit was subsequently investigated in some detail by Chittenden [5], and his results have recently been published. The ferment exists in the unaltered juice of the pine-apple, which is found to have a profound and rapid digestive effect on such bodies as fibrin and egg-albumin, converting them into proteoses and peptones with formation of both leucin and tyrosin. It is hence a tryptic ferment like papaïn. The powers of the juice are seen best in a perfectly neutral solution, a little acid quickly diminishing its activity.

[1] Compt. Rend. July 1880.
[2] Biol. Centr. 1884, also Arb. d. bot. Inst. in Würzburg, iii. 1885.
[3] Ann. of Botany, vol. VI, 1892.
[4] Bulletin of Pharmacy, vol. V, p. 77, 1891.
[5] Trans. of Connecticut Academy, vol. VIII, 1891.

The acidity of the natural juice is equal to about an acidity of ·5 per cent. HCl ; the proteolytic powers in such a juice compared with those in a neutralised one being about as 3 : 4. Alkalinity is also harmful, ·5 per cent. Na_2CO_3 hindering the decomposition of the proteid, and 1 per cent. inhibiting it altogether. The ferment, if freed from the salts, &c. present in the natural juice, by precipitation by alcohol and subsequent solution in water, is still more sensitive to acid, being quite without effect in the presence of ·1 per cent. HCl.

The temperature at which it has the greatest activity also varies with the reaction. The natural juice works best at 40° C., and is stopped and the ferment destroyed at 70°. The ferment in the neutralised juice continues active at this temperature, and is not destroyed under 80° C., its optimum being between 50° and 60° C.

The ferment can be separated from the juice by several methods, but none yield it pure, the proteids of the juice being thrown down with it. It can best be precipitated by saturating the juice with Na Cl or $MgSO_4$: less advantageously by saturation with sulphate of ammonium, or by about 80 per cent. of alcohol.

The part that these four proteohydrolytic enzymes play in the metabolism of the plants in which they occur is not very evident. The probability that such bodies have a good deal to do with the processes of germination soon occupied the minds of botanists, and such seeds as store quantities of reserve proteids in their tissues were the subjects of research for some years after the discovery that the ferments existed. In 1874, von Gorup-Besanez [1] detected an enzyme in the seeds of the Vetch, which has the power of forming peptone from fibrin, and in 1875 he made known the existence of the same body in the seeds of Hemp, Flax, and Barley [2]. He did not indicate, however, what its action is on the reserve proteids of the seeds. In 1878 Krauch [3] criticised adversely his methods

[1] Ber. d. deutsch. Chem. Gesells. 1874, p. 1478. [2] Ibid. 1875.

[3] Beiträge zur Kenntniss der ungeformten Fermente in den Pflanzen, Berlin, 1878.

of working and denied the accuracy of his results. There seems from later researches no doubt however that von Gorup-Besanez was in the main correct.

In 1887 the writer published[1] an account of some investigations into the germinative processes of the Lupin which established the existence of von Gorup-Besanez's enzyme, and which pointed out the nature and conditions of its action, as well as the value of it to the plant. The enzyme does not exist as such in the resting seed, but makes its appearance at the onset of germination. A very active extract can be prepared from seeds which have been germinated four days. The fleshy cotyledons, if ground and soaked with glycerine for a few hours, give up to the solvent a quantity of the ferment, which can be purified from the products of its activity by dialysis. It works, unlike papaïn, most advantageously in an acid medium, the degree of acidity most favourable being ·2 per cent. HCl, which is a little more intense than the reaction of the germinating seed itself. It will not act in the presence of alkalis, even though very dilute, and neutral salts impede it. It is utterly destroyed by not very prolonged contact with even dilute alkalis. Like other ferments it is destroyed by boiling.

When a dialysed glycerine-extract is allowed to act on fibrin in a parchment-paper dialyser kept at a temperature of 40° C., the extract and the external fluid being both kept acid to the extent of ·2 per cent. HCl, the dialysate soon contains peptone and leucin and tyrosin. In the digestion-tube there can be found, besides the undigested fibrin, a certain amount of acid-albumin, and some albumoses. The ferment, like the ferment of pancreatic juice, apparently decomposes the fibrin first into acid-albumin and proteoses, and later these give rise to peptone, leucin, and tyrosin, following the course of proteolysis suggested by Kuhne. Like the animal trypsin, the process is rather one of corrosion than of solution. The digestion contains, however, more proteose than is formed by

[1] Green, On the changes in the proteids in the seed which accompany germination, Phil. Trans., vol. 178 (1887), B. p. 39.

the latter enzyme. The proteose is really a mixture of two bodies, corresponding fairly well with Kuhne's hetero- and dys-albumose.

When the proteids of the seeds are used instead of fibrin, the course of the digestion is similar; acid albumin, peptone, and amide-bodies are produced, the latter including asparagin, which does not occur in the digestion of fibrin.

The value of it to the germinating seed is therefore its power to convert the stored proteids of the latter into such bodies as can readily pass out of the cells in which the proteids are deposited, and make their way to the growing parts of the young seedling.

Besides the Lupin, this ferment is found to occur in the endosperm of germinating seeds of *Ricinus communis*, the Castor-oil plant [1].

Another proteohydrolytic ferment was described in 1892 by Daccomo and Tommasi [2] as obtainable from *Anagallis arvensis*. It can be isolated under the form of a white amorphous substance, easily soluble in water. If the fresh plant be reduced to power and kept in contact with fresh meat or fibrin at a temperature of 60° C. for four or five hours, the authors say that it is considerably softened, though complete disintegration is not effected in less than thirty-six hours. The ferment is stated to have the property of destroying fleshy growths and horny warts.

Rennet. Another ferment, which, from its resemblance to the rennet of the animal organism, may be presumed to belong to the class of proteohydrolysts, has been noted by many observers as being widely distributed in the vegetable kingdom. Its occurrence is much like that of the peptic and tryptic classes, it being found in very various parts of different plants. Prior, in his Popular Names of British Plants, speaks of a curious property of *Galium verum*, which was noted by Matthioli in the sixteenth century, who wrote of it, 'Galium inde nomen sortitum est suum quod lac coagulet.' Even now in the West of England

[1] Green, Proc. Roy. Soc. vol. XLVIII, p. 377.
[2] Abs. in Rev. de Therap. LIX, p. 470.

it is the custom of dairymen to put this plant into milk to set the curd ready for cheese-making. The active principle seems to be located in the flowers, though the whole plant is used.

The power of curdling milk was stated by Linnæus[1] to exist in the leaves of *Pinguicula vulgaris*, which he says were used for that purpose by certain Lapland tribes. Pfeffer says that they are also used in the Italian Alps to the same end. Darwin noted that the secretion of the glands of *Drosera* had the same power[2]. The latex of *Carica Papaya*, the bast of the stem of *Clematis Vitalba*, and the petals of the artichoke (*Cynara Scolymus*), also curdle milk, when allowed to remain immersed in it.

The ferment has been extracted in recent years from a large number of seeds, some before and others during germination. The fullest account of its properties has been given by Lea[3], who prepared it from the resting seeds of *Withania coagulans*, a shrub which grows freely in Afghanistan and Northern India. *Withania* is a genus of the natural order Solanaceae, and has a capsular fruit, containing a large number of small seeds. From these it can be extracted either by glycerine or by a moderately strong solution of common salt. It is destroyed by boiling, but it can withstand a moderately prolonged exposure to alcohol. Its activity is about the same as that of most commercial samples of animal rennet.

Martin[4] has shown that commercial papaïn contains rennet, but he does not speak of its situation in the plant.

During the last few years the writer has met with vegetable rennet in the seeds of *Datura Stramonium*, *Pisum sativum*, *Lupinus hirsutus*, and *Ricinus communis*[5], in the two former in the resting, and in the two latter in the germinating condition. In *Ricinus* it does not exist in the resting state, but the seed will then give up to an appropriate solvent a principle in which the milk-curdling power can be developed by warm-

[1] Flora Laponica, 1737, p. 10.
[2] Insectivorous plants, 2nd edn., p. 94.
[3] Proc. Roy. Soc. 1883.
[4] Journal of Physiology, VI, p. 340.
[5] Green, On the germination of the Castor-oil plant, Proc. Roy. Soc., vol. XLVIII, p. 391.

ing with dilute acids. From the endosperm of germinating seeds the enzyme can be extracted by either salt-solution or glycerine. It is associated with the trypsin already mentioned, as well as with another ferment to be described presently. The enzyme is often present in good quantity, or it has very energetic powers, a glycerine-extract in one experiment curdling two and a half times its volume of milk in five minutes. The salt-solution extract acts much more slowly, neutral salt being a hindrance to rennet, as it is to trypsin. Different seeds, however, contain very varying quantities of the enzyme.

In the germinating lupin-seed, rennet exists side by side with trypsin, but there is much less of it present.

The rennet from *Ricinus* is capable of acting in either acid, neutral, or alkaline solutions. Too much acidity obscures the action, as the acid itself tends to throw down the casein of the milk.

The so-called 'Naras' plant of South Africa [1] (*Acanthosicyos horrida*) also contains rennet in the pericarp, in the pulp, and in the expressed juice, of its ripe fruit. It differs from the examples just quoted in not having any in the seeds. The enzyme in Naras is destroyed by boiling, but it will remain for an almost indefinite time in the dried rind. It differs from most ferments, according to Marloth, in being soluble in alcohol of 60 per cent. strength.

Chittenden's proteohydrolytic enzyme in the pine-apple is also associated with a rennet-ferment [2].

Glyceride-Enzymes.

The transformations undergone by oils on the germination of the seeds containing them have only recently been shown to be the work of an enzyme. In 1871 Müntz [3] showed that during germination bodies make their appearance that are such as would result from a splitting up of the oil, and suggested that the embryo acts as a ferment and provokes the decomposition. Schützenberger [4] in 1875, from observations on seeds crushed

[1] Nature, July 19, 1888, p. 275.
[2] loc. cit.
[3] Müntz, Annales de Chimie, sér. 4, vol. XXII, 1871.
[4] op. cit.

in water, suggested that they contain a saponifying enzyme. Such a ferment was described by the writer in 1890[1], as occurring in the seeds of *Ricinus communis* during the period of germination. It can first be identified after a few days of that period have passed, and the endosperm is seen to be in process of absorption. From such enlarged and swollen endosperms an extract should be prepared by soaking them for twenty-four hours either in glycerine or a solution of common salt containing .5 per cent. NaCl, with a trace of some antiseptic such as KCN to prevent putrefaction. If the latter solvent be used, when the liquid has been strained from the pulp and filtered, it should be dialysed for some time to get rid of the greater part of the salt, as this impedes the action of the enzyme. The extract will then be slightly opalescent or nearly clear, and will contain a little proteid matter, coagulating on boiling.

When such an extract is mixed with twice its volume of an emulsion of castor-oil (which should be made as thick as possible to approximate to the conditions obtaining in the cells of the seed), and the mixture is exposed to a temperature of 40° C. in an incubator, an acidity is soon developed in the liquid, which the addition of a few drops of litmus-solution at once makes evident. If the operation be carried out in a dialyser, the liquid outside the membrane does not share the acidity, showing that the latter condition is due to something that cannot diffuse out of the dialyser. This body can be extracted from the contents of the latter by shaking them up with ·2 per cent. NaHO, and filtering. If the resulting alkalinity be neutralised with a mineral acid, a quantity of fatty acid soon rises as a scum to the surface.

If the digestion be allowed to go on in the dialyser for some days, glycerine can be detected in the dialysate.

If the extract be boiled before mixing with the emulsion no such decomposition takes place. The transformation is therefore shown to be due to the action of an enzyme, capable of splitting up oil into fatty acid and glycerine.

[1] Green, On the germination of the Castor-oil plant, Proc. Roy. Soc., vol. XLVIII, 1890, p. 370.

Müntz's suggestion that the embryo acts as a ferment is not borne out by the facts. Neither the cotyledons nor the axis of the embryo contains any enzyme, the latter being present in the cells of the endosperm only. Its distribution is not limited as is that of the ferments in the germinating barley-grain, but extends throughout the whole endosperm, the part nearest the cotyledons not being at first more attacked than the rest, though the absorption of the reserve materials by the embryo begins there and leads to the gradual destruction of the endosperm from that side.

In the resting seed of *Ricinus* this ferment exists in the condition of a zymogen; which can be transformed into the active enzyme by the action of weak acids at 45° C. for about three hours, or by the prolonged action of water at the ordinary temperature.

The ferment, like so many of the others described, is found to be very sensitive to changes in the reaction of the medium in which it is working. It is most active in a neutral medium, is hindered by ·066 per cent., and stopped by ·133 per cent. HCl. With alkalis the hindering effect is not so marked, ·066 per cent. of Na_2CO_3 only retarding it slightly. A little less than 1 per cent. is quite inhibitory. The ferment is not destroyed by the action of these reagents, for on neutralising the solution it resumes its activity. It is, however, much more readily damaged by acid than alkali, exposure to ·133 per cent. HCl for three and a half hours reducing its activity nearly 90 per cent., while ·66 per cent. Na_2CO_3 in the same time only lessens its powers one-half.

The existence of fat-splitting enzymes has since been demonstrated by Sigmund[1] in both resting and germinating seeds of the Rape, the Opium Poppy, Hemp, Flax, and Maize. His mode of experiment was to crush the seeds with water, and estimate the free fatty acid in the resulting emulsion immediately, and again after allowing it to stand twenty-four

[1] Sigmund, Ueber fettspaltende Fermente im Pflanzenreiche. Sitzungsber. d. k. Akad. der Wissensch. Wien, Math.-Nat. Classe, Bd. XCIX, July 1890, and Bd. C, July 1891.

hours at a temperature of about 30° C. He found that the resting seed contained a certain amount of enzyme, and that this was increased at the onset of germination.

Enzymes of Fungi.

The ferments existing in the lowlier plants have only within recent years come to be regarded as corresponding to those so far described. The old division into organised and unorganised ferments was held to be a very sharp and well-defined one, and all the lower Fungi were classed with the former, whatever their mode of action. That this view was not well founded is evident from the facts that are detailed above as to the existence of isolable invertase in Yeast and *Fusarum*, and of the cytohydrolyst in *Botrytis*. To this point, however, we shall return shortly. Another member of the yeast-family contains a ferment which can be separated from it by appropriate treatment, and which therefore weakens further the old distinction. This is the so-called *Torula Ureae*, which flourishes in solutions of urea or in putrefying urine, on which it appears to subsist, decomposing the urea with formation of ammonium carbonate. It was first investigated by Müller[1], Pasteur[2], and Van Tieghem[3], and later by Musculus[4], and more completely by Lea[5]. The latter observer obtained from fermenting urine a copious development of the *Torula*, and found that from the cells he was able to isolate an active principle which was capable of decomposing urea in the way the unaltered *Torula* did. The urine, with its contained organisms, was precipitated by strong alcohol, dehydrated and dried. A little of the resulting powder introduced into a 2 per cent. solution of urea and kept at 38° C. gave an alkaline reaction in a few minutes, and very soon caused a powerful odour of ammonia to be noticed. The precipitate when treated with distilled water gives the enzyme up to it, for when the undissolved matter, consisting chiefly of the cell-bodies, mucus

[1] Journ. f. prakt. Chem. Bd. LXXXI, 1860, S. 467.
[2] Compt. Rend. t. L, p. 869. [3] Compt. Rend. t. LVIII, p. 210.
[4] Compt. Rend. t. LXXVIII, p. 132, and Ibid. t. LXXXII, p. 333.
[5] Journal of Physiology, vol. VI, p. 136.

from the urine, &c., is removed by filtration, the clear slightly alkaline filtrate can decompose the urea just as the dried powder can. By repeated solution and precipitation the enzyme can be separated in a fairly pure condition, when it appears as a white powder, soluble to a clear solution in distilled water, and giving a very faint xanthoproteic reaction. Its activity is destroyed by heating to 80–85° C.

If the original urine be filtered before the first precipitation by alcohol, the filtrate contains no ferment, the enzyme residing entirely in the cells. Until these are destroyed by the spirit, the enzyme cannot be extracted, being apparently unable to diffuse through the protoplasm and cell-wall. The same peculiarity, it may be observed, is characteristic of the *Torula* yielding invertase, which can only be extracted after the death of the organism [1]. The action is an intracellular one only, the urea being absorbed and the ammonium carbonate excreted.

The action of the ferment of *Torula Ureae* is, like most others, one of hydration; it seems to be concerned in the active life and nutrition of the plant.

The Enzymes of Bacteria.

In recent years several observers have been able to extract from various other micro-organisms, especially bacteria, enzymes which may be said to belong to one or other of the different groups described. In 1887, Bitter showed that certain of these forms produced some that could be separated from the microbes themselves. He killed the organisms by sterilisation at 60° C., and ascertained that that temperature did not destroy the enzymes, which continued able to liquefy gelatin and to peptonise albumin. Hankin extracted from the bacillus of anthrax an enzyme that is capable of forming albumoses from fibrin. Several toxic bodies of this class have been traced to similar agency, an extract prepared from the organisms being capable of forming them in the absence of the cells. The ordinary putrefactive bacteria may excrete or yield an enzyme resembling trypsin in

[1] Lea, loc. cit.

its action on proteids[1]. Sirotinin showed that culture-fluids t
had been filtered through porcelain could still liquefy gelat
The same microbe may secrete more than one enzyme,
preponderating formation depending on the nature of
medium in which it is cultivated. Thus Lauder Brunton a
MacFadyen[2] isolated two such bodies from the same micro
one of a peptonising nature appearing most prominent when
bacillus was cultivated in meat broth, and a diastatic one wh
the culture-medium was starch-paste. These were two enzym
and not one with both powers; the former being most eas
extracted. Acid favoured and alkalis impeded its activity.
Wood[3] also identified two enzymes in each of four microb
These were Koch's cholera-bacillus, Deneke's cheese-bacill
Finkler's cholera-nostras-bacillus, and Miller's bacillus. T
two enzymes were a peptic and a rennet ferment, and w
prepared from sterilised culture fluids in which the vari
microbes had grown. Wood found that the enzymes from t
different bacilli varied a good deal in their power of resisti
the influence of acid media, those from Koch's bacillus bei
destroyed by very little acidity, while those from Finkler's a
Miller's bacilli could act in distinctly acid solutions. The two
zymes themselves showed a different power of resistance, a c
gulation of casein occurring when peptonisation was complet
inhibited. With carbolic acid the effect was exactly the reve
the rennet being destroyed before the proteo-hydrolytic o
Wood noticed that the bacilli themselves showed a varying s
ceptibility to acids exactly corresponding to that of the enzym

When the cholera-bacillus is cultivated on starch-paste
can liquefy it and form sugar, but this power is not like th
of coagulating milk and peptonising proteid, as it cannot
extracted from the cells, appearing to reside wholly in t
protoplasm. Wortmann[4] has ascertained that certain bacte

[1] Hüfner, Journ. f. prakt. Chem. Bd. V, 1872, S. 872. Hermann, Ztschr. f. phys
Chem., Bd. XI, 1887, S. 523. Salkowski, Ztschr. f. Biol., Bd. XXV, 1889, S.
[2] Proc. Roy. Soc. XLVI, 1889, p. 542.
[3] Laboratory reports, Roy. Coll. Phys., Edinburgh, vol. II.
[4] Wortmann, Zeitschr. f. Physiol. Chem. VI, 1882, p. 287.

exert diastatic powers on starch through excreting an enzyme when starch-grains are their only available food. *Bacillus Amylobacter* [1] breaks up cellulose by the same process, forming bodies which are soluble in water. According to Fitz and Hueppe [2], the same bacillus excretes a rennet-enzyme when cultivated in milk.

The most remarkable of these microbes is *Bacillus mesentericus vulgatus*, which Vignal [3] has shown to contain at least five separate enzymes; diastase, invertase, rennet, a proteo-hydrolytic one, and one dissociating vegetable cells by destroying the middle lamella. Though these all can be extracted from the microbe, the proportions vary much according to the culture-medium.

The possession of several enzymes by the same cell seems at first rather strange, but we find the same thing in multicellular plants. Thus the germinating lupin-seed forms three enzymes in the cells of its cotyledons—rennet, diastase, and trypsin; the castor-oil seed contains rennet, trypsin, and a glyceride-enzyme. All appear to originate in these two cases in the same cells. The animal organism also shows pepsin and rennet existing together in the peptic cells of the stomach, and three ferments in those of the pancreas.

The cells of the cholera-bacillus, as mentioned above, behave very similarly to their enzymes with regard to their resistance to acids. They show a similar correspondence as to their optimum temperature for activity.

The influence of the mode of cultivation on the microbe in the formation of the enzymes has been the subject of research, but no very complete investigation has at present been made. Flugge [4] has shown that if oxygen be prevented access to them during their growth, they do not excrete enzymes.

A suggestion has been made by Wood, in his paper referred to above, as to the reason for the secretion of enzymes by the bacilli he examined. In the absence of such a secretion the

[1] De Bary, Lectures on Bacteria, p. 69 and 101.
[2] Contribution à l'étude des bactériacées (Thèse pour le doctorat-ès-sciences naturelles, Paris).
[3] Ibid. p. 104.
[4] Die Micro-organismen, 1886, p. 470.

protoplasm must be brought into as close contact as possible with the nutrient medium in which the microbe is growing. Its extreme susceptibility to acids renders this rather a hindrance to the multiplication of the bacillus. An enzyme excreted and effecting the changes in the medium without such close contact with the organism enables the latter to secrete a firmer and more resistant cell-wall, thereby protecting it from adverse influences. He bases this hypothesis on noting the effects of acids on the organism when grown anaërobiotically, or without secreting the enzyme, and when cultivated under normal conditions.

The enzyme in most cases is more resistant to the so-called antiseptics than is the bacillus producing it. Most of these antiseptic bodies will enable an active ferment-extract to be prepared while the organism is kept from developing. Wood shows that the cholera-bacillus is an exception, one to two drops of a 5 per cent. solution of carbolic acid in 10 cc. bouillon destroying the enzyme but not damaging the organism.

Zymogens.

Most of the enzymes hitherto described have only been investigated with regard to their distribution and behaviour, their mode of formation being left undecided. Analogy with similar bodies occurring in the animal organism suggests that they originate in the condition of zymogen, or mother of ferment. So far as histological evidence is available, their appearance in the cells is strikingly like the corresponding process in animal cells, pointing to their formation as granules from the protoplasm.

The existence of vegetable zymogens was first established by Vines[1] in his experiments on *Nepenthes*. He treated some pitchers of this plant with dilute acetic acid (1 per cent.) for twenty-four hours before extracting them with glycerine, and at the same time extracted other similar pitchers with glycerine without preliminary treatment with acid: the first extract possessed greater powers of digestion than the second, leading him to infer that, as in the secreting cells of the stomach and

[1] op. cit.

pancreas, a zymogen is present in the glands of *Nepenthes* which was converted into enzyme by the action of the acid.

The writer's experiments on the antecedent of the enzyme in the resting-seed of the Lupin [1] also indicate a similar condition in the cells, though its identification is not so easy, as the acid treatment usually adopted for zymogen conversion is not available. It was ascertained to exist as the latter by a method adopted by Langley and Edkins [2] in their researches on the relation of pepsinogen and pepsin in the gastric cells.

Inulase can be more easily shown to exist as a zymogen in the resting artichoke-tuber. One method of preparing an active ferment from the pancreas is to take the fresh gland containing no trypsin and to keep it for some hours at a temperature of 40° C. when it yields a considerable quantity. Some pieces of full-grown artichoke-tubers were treated in this way, being kept at 35° C. for twenty-four hours. An extract prepared from them then was found to convert inulin into sugar, while an extract made from other pieces of the same tubers without warming was inert. When some of this latter extract was warmed for a time with a solution of acid-albumin in ·2 per cent. HCl, some ferment was developed in it, though less than was yielded by warming the tubers alone before extraction as just described. The free-acid treatment alone was not applicable in the case of inulase, as the quantity of acid needed to convert the zymogen was sufficient to destroy any ferment liberated from it.

The glyceride- and rennet-ferments of the castor-oil-seed were also shown by the acid-method to exist in the zymogen-condition until the onset of germination, the former of them being convertible into the ferment also by the prolonged action of water [3].

Brown and Morris [4] mention that the secretion of diastase by the epithelium of the scutellum of barley is increased 20 per cent. by the addition of very dilute formic acid. Baranetzky found that a freshly-prepared extract of the

[1] op. cit. p. 50.
[2] Journal of Physiology, vol. VII, pp. 371–415.
[3] Green, op. cit.
[4] op. cit.

leaves of *Melianthus major* was inactive on starch, but that after standing a few days it had diastatic powers. He noted the same thing in the case of potato-tubers.

Experiments of Reychler[1], and of Lintner and Eckhardt[2], point to the existence of a zymogen in the cells of the grain of wheat. They found that the action of a dilute acid upon the gluten of wheat gave rise to a diastatic enzyme. Frankhause[3] found further that in germination of barley small quantities of formic acid could be detected in the grains. This may be regarded as important in discussing the increase of diastatic power attending germination, as it would probably transform zymogen into ferment.

Though the results of histological examination of the cells in which the various enzymes occur are not at all complete, they point, so far as they go, to a similar mode of secretion to that obtaining in animal cells, and hence indirectly to an antecedent zymogen. Gardiner[4] has described the changes in the cells of *Dionaea muscipula* in the states of rest and activity: in the first condition the cells show a very granular protoplasm lining the cell-wall, leaving a single central vacuole, the granules being so numerous as to obscure the nucleus which lies at one end of the cell. After stimulation the leaves begin to secrete and continue to do so for twenty-four hours. In this second condition, that of activity, the protoplasm has lost its granularity, being clear and hyaline; the nucleus has come to occupy the centre of the cell and strands of protoplasm connect it with the peripheral layer. The same observer shows that the gland-cells of *Drosera* in the resting state are much more granular than they are after secretion.

Brown and Morris[5] describe similar changes in the cells of the epithelium of the barley-scutellum during the early stages of germination. When this process begins, the protoplasm,

[1] Reychler, Ber. 22, 414.
[2] Lintner und Eckhardt, Zeitschr. für das gesammte Brauwesen, 1889, p. 389.
[3] Frankhause, Der Bund. Berne 37, No. 26.
[4] Proc. Roy. Soc. 1883.
[5] op. cit. p. 467.

originally very finely granular and semi-transparent, becomes much coarser in appearance, the granules increasing to such an extent as to make the nucleus almost invisible. When the endosperm is becoming exhausted of its reserve material, the cell-contents clear again and become even more transparent than they were at first. The process of secretion in these epithelium-cells continues so long as they are supplied with a flow of nitrogenous material from the endosperm across the epithelium.

Marshall Ward [1] also calls attention to the brilliant refringent granules in the cytohydrolytic drops obtained from the hyphae of *Botrytis*, which appear to be secreted from the protoplasm, as evidenced by their giving proteid reactions. These granules only appear when the hyphae are secreting the enzyme. Guignard [2] points out the granular character of the cells secreting myrosin in the root of the horse-radish. Similar granules occur, according to Marshall Ward and Dunlop [3], in the cells of the raphe of the seeds of *Rhamnus infectorius*, in which they located the rhamnase of that plant. The same granular character occurs in the cells of the epithelium of the haustorium in *Phoenix* and other Palms.

Constitution of the Enzymes.

There has been much speculation as to the nature of the enzymes and their zymogens. From the fact of their secretion directly from the protoplasm and the histological changes observed in the latter during the secretory process, the opinion has been advanced that the zymogens are proteids from which the enzyme arises by a decomposition consequent on oxidation [4]. Heidenhain [5] suggested that the zymogen consisted of the ferment in combination with an albuminoid body. By many observers the suggestion has been made that the enzymes themselves are proteid bodies. Loew holds that they are proteids allied to the peptones, basing his opinion upon analysis of them [6]. In

[1] op. cit. [2] op. cit. [3] op. cit.
[4] Vines, Physiology of Plants, p 193.
[5] Pflüger's Archiv, 1875, Bd. X, p. 581.
[6] Pflüger's Archiv, XXVII, 1882.

support of the hypothesis of their proteid nature we have the fact that when an extract containing an enzyme has all its proteids removed by precipitation, the filtrate possesses little or no ferment power, the latter being diminished in many cases in proportion as the proteid is thrown out of solution. This may, however, only indicate that the ferment is precipitated with the proteid, and indeed it is known that quite inert precipitates can carry enzymes out of solution with them, a fact taken advantage of by Brücke in his process for preparing pepsin. A more striking fact is that the temperatures at which so many enzymes are destroyed correspond very closely to the points at which proteids occurring with them are coagulated. Chittenden calls attention to this point in his work on the pine-apple [1], and it has been noticed by many writers on the animal ferments.

On the other hand, we have considerable evidence to show that though associated with proteids in the cells, there is no identity between them and the latter. Hartley has shown that at least some of them do not act upon the spectrum in the same way as proteids do; chemical analysis too shows them to contain less nitrogen than the latter bodies. Too much stress should perhaps not be laid upon this fact, as we have no evidence that the bodies analysed were the pure enzymes. Evidence against their being proteids can be deduced from the modes of preparation which in some cases have been found to yield them in very active condition, and which present them in solutions giving very slight, if any, proteid reactions. Brücke's method of preparing pepsin from the stomach shows clearly that this at least is not a proteid. We must admit, however, that they are in some way very closely attached to such bodies, particularly to certain members of the globulin-group.

In recent years one ferment has been the subject of critical examination. O'Sullivan and Tompson, in their paper already quoted, have published an account of a long series of experiments that they carried out with a view to ascertaining the

[1] op. cit.

composition of invertase, in the course of which they succeeded in obtaining it perfectly pure, and in investigating the products of its decomposition. They conclude from their investigations that this enzyme is a member of a homologous series of bodies to which the name *the invertan-series* is given. The series consists of seven members, α, β, γ, δ, ϵ, ζ and η invertan, which differ from each other in the proportion of nitrogen which they contain and in their optical activity. The highest member of the series, α *invertan*, is a more stable body than the remainder and is insoluble in water. The remaining six are freely soluble, the solutions being clear but rather viscous; they do not coagulate on heating. They all agree in being colourless when dissolved, in not dialysing and not crystallising. When alcohol is added to alkaline or neutral solutions the latter become milky and the cloudiness is not removable by filtration. Acids added to the milky liquid cause precipitation. With copper-oxide in alkaline solution, and in the presence of caustic potash, they all give precipitates which are very bulky and almost mucilaginous. Invertase itself, the authors consider to be the second member of the series, β *invertan*, and on its decomposition it splits up into the first and fourth members, the former containing more and the latter less nitrogen than invertase. Their views of the composition of the members of the series are, that with the exception of the lowest they are all combinations of a peculiar proteid yielded by the yeast-cells, to which they give the name *yeast albuminoid*, with the lowest member, η *invertan*, and that the latter body is itself probably a combination of the same proteid with a carbohydrate, eighteen parts by weight of the latter uniting with one part of the albuminoid. A full discussion of their views will be found in their paper alluded to [1].

Action of the Enzymes.

The action of these enzymes seems in nearly all cases to be one of hydration, myrosin so far being the only exception. This is undoubtedly the case in the simplest transformations.

[1] Journ. Chem. Soc., No. CCCXXXV, Oct. 1890, p. 835.

Thus invertase sets up the action expressed by the equation (p. 92) $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$.

Emulsin (p. 99)

$$C_{26}H_{27}NO_{11} + 2H_2O = C_8H_5COH + HCN + 2C_6H_{12}O_6;$$

the glyceride-ferment (p. 114)

$$\underset{\text{olein}}{C_{57}H_{104}O_6} + 3H_2O = 3\underset{\text{oleic acid}}{C_{18}H_{34}O_2} + \underset{\text{glycerine}}{C_3H_5(HO)_3};$$

the enzyme of *Torula Ureae* (p. 117)

$$\underset{\text{urea}}{CON_2H_4} + 2H_2O = \underset{\text{ammonic carbonate.}}{(NH_4)_2CO_3}.$$

The action of diastase has been variously stated by different authors, but all agree that the process is one of hydration. The transformation brought about by the cytohydrolytic enzyme has not been fully followed out, but it undoubtedly leads to the production of some form of sugar. There is a certain amount of evidence obtainable from a study of the germination of the Palms, indicating successive hydrations of the cellulose prior to its disappearance.

The relations of the proteids to the peptones and amides springing from them is a much more difficult matter to deal with. The knowledge we possess of the chemical constitution of a proteid is so small that it is difficult even to speculate on the nature of the changes which it undergoes in digestion, while its molecule is so large that the possibility of its taking up water in such changes almost escapes the power of analysis. Several views have been advanced as to the relation of ordinary proteids to peptones, the chief being that this is either one of hydration, or that proteids are polymers of peptones. In favour of the former view we have analogy with the majority of the enzymes known, and the fact that peptone agrees with the hydrated products of the latter in increased solubility in water as compared with ordinary proteids. Moreover it is possible by dehydrating agents to convert peptone into a body resembling syntonin, which is itself an intermediate product formed during the conversion of albumin or globulin into peptone. If 10 parts of dry peptone

be taken and mixed with twice their weight of acetic anhydride, the mixture then heated for a long time to 80° C., and finally the excess of acetic anhydride distilled off and the residue dialysed, it is found to be changed into a proteid that is not diffusible, is soluble in dilute alkali, is precipitated by acetic acid and potassic ferrocyanide, and by many metallic salts, as ordinary proteids are. According to Hofmeister a similar effect may be produced by prolonged heating to 140° C. The resulting brown mass contains a part soluble in water and another not so, which react after the manner of a globulin and a derived albumin respectively.

Other views of the relationship have been advanced, some observers believing that peptones are isomers of proteids. Adamkiewicz suggests that they differ in the removal of salts, and a re-arrangement of the molecule. The most recent hypothesis was put forward by Schützenberger [1] last year. He holds that peptone is a mixture which, by treatment with phosphotungstic acid, can be separated into two parts, one containing a little more oxygen than the other, and both being ureïde bodies. Fibrin, on the other hand, is a kind of compound ether, which is saponified by the enzyme and in taking up water splits into the two bodies found. The transformation is thus one of hydration, being the result of the decomposition of an ether by saponification.

The action of an enzyme appears to differ in no way from an ordinary chemical reaction. Most of the changes that are brought about by such bodies can be effected in the laboratory by ordinary chemical processes, starch being hydrolysed to sugar by dilute mineral acids, fats split up by alkalis or superheated steam, peptones formed by heating proteids to high temperature in a Papin's digester. Invertase has been specially investigated by O'Sullivan and Tompson [2], who find that the rate of inversion of cane-sugar by it may always be represented by a definite time-curve which 'is practically that given by Harcourt as being the one expressing a chemical change of which no condition varies excepting the diminution

[1] Comptes rendus, CXV, p. 768.

[2] op. cit. p. 926.

of the changing substance.' J. O'Sullivan also finds that the hydrolytic action of yeast at the ordinary temperature follows the same course as that of a simple chemical interchange, while its rate differs from that at which the alcoholic fermentation of yeast takes place. The fact that the action of the enzymes is always accompanied by an evolution of heat is also evidence to the same end. Lea[1] states, on the authority of Hoppe-Seyler and other observers, that the heat of combustion of the products of zymolysis is in all cases less than that of the substances from which they are derived.

That we have to do then with an ordinary chemical reaction leading to hydration and subsequent decomposition, seems clear. What the exact nature of that reaction must still be largely a matter of hypothesis. The first thing that strikes an observer in this connection is the extremely small amount of the enzyme that is needed to bring about the transformation of an enormous amount of the body which it attacks. Thus O'Sullivan and Tompson[2] show in one of their experiments that a sample of invertase induced inversion of 100,000 times its own weight of cane-sugar and that it was not destroyed or even injured by its action. This latter feature, which was first determined by Foster[3] in the case of salivary diastase, has been shown by many other observers to be characteristic of other enzymes. Based upon these two considerations we have the hypothesis that enzymic action may be similar to the action of nitric oxide in the manufacture of sulphuric acid. Thus the ferment may be regarded as carrying water to the initial body by uniting with the latter, the combination now being capable of taking up water and being thereby decomposed. The resulting products may be only the hydrated body, or a number of bodies, while in all cases the decomposition liberates the enzyme unaltered. The decompositions we have seen are usually complex, diastase giving rise to various dextrins and maltose; trypsin

[1] The Chemical Basis of the Animal Body, p. 75, 1892.
[2] op. cit. p. 927.
[3] M. Foster, On Amylolytic Ferments, Journ. Anat. and Phys., vol. I, 1867, p. 107.

to proteoses, peptones and amides; emulsin to several bodies of which glucose is one. Though there is but little direct evidence to establish this hypothesis, certain facts that have been noticed lend a certain support to it. O'Sullivan and Tompson [1], in tracing the action of heat on invertase, found that this varies greatly according to the presence or absence of cane-sugar in the experiments. When no cane-sugar was present, the enzyme was almost all destroyed by heating to 50° C., while in its presence this effect was not produced till the temperature was 75° C., a difference of 25° C. The authors advance as a possible explanation the view that the invertase enters into combination with the sugar, and that the resulting body can resist the heat more successfully than the invertase alone. They hold that the combination is broken up when the compound molecule meets with another molecule of cane-sugar. A fuller discussion of their hypothesis will be found in their paper already alluded to [2].

Bearing on the same point is Chittenden's observation [3] that if neutralised pine-apple juice be heated to 60° C. in the absence of any proteoses or peptones, the ferment is rapidly destroyed, whereas this temperature is the one at which the enzyme is most active if proteids be present during the heating. Biernacke [4] found similarly that albumoses or peptones raised the temperature at which trypsin is destroyed by five degrees or more, and that while pepsin in the absence of peptone was destroyed in acid solution by a temperature of 55° C. it was active after being heated with peptone to 70° C.

We may note in this connection too the possible significance of the inhibitory effects of traces of acid or alkali in the solution in which the enzyme is working. The minute trace of the reagent seems to correspond to the extremely small amount of the enzyme usually present, and it is at least possible that it may work by entering into some combination with the latter, the body formed not being capable then of uniting with the substance which the enzyme would ordinarily

[1] op. cit. p. 900. [2] op. cit. p. 919. [3] op. cit. p. 17.
[4] Zeitschr. für Biol., Band XXVIII, p. 49.

transform. That acids and enzymes do unite we know from a consideration of the relations between pepsin and hydrochloric acid, the ferment being absolutely inoperative without the acid. Biernacke has shown that when pepsin is heated in the presence of ·2 per cent. of HCl to 60° C. it is destroyed, and that the same destruction is reached 5° C. lower if no acid be present. He noted a similar relation between trypsin and an alkali. Chittenden found that the trypsin of the pine-apple will stand a higher temperature in neutral than in acid solution. In this case the compound, if it exists, is less stable than the enzyme alone.

Reviewing the actions of these various ferments, it is apparent that they may be divided into two classes. One set act only intracellularly and do not during their activity leave the cells in which they are secreted. As examples of these we have the ferment of the lupin, the invertase of yeast, the urea-decomposing ferment of *Torula Ureae*, and probably the diastase of translocation. The others are secreted in particular cells and are excreted by them to work upon substances contained elsewhere. Such are for example the ferments in the epithelium of the scutellum of the germinating barley-grain, the glucoside-ferments described by Guignard and by Marshall Ward, the ferments of *Drosera* and other carnivorous plants.

The power of diffusion which these enzymes possess is very slight. When extracted from the plants and subjected to dialysis in ordinary vessels with parchment septa they cannot pass through the wall of the dialysers ; they are able however to make their way through the cell-wall of the cells in which they are secreted. This need not be a matter of surprise when we consider the extreme tenuity of the film composing the wall, which cannot be approached by any membrane we can use in laboratory experiments. The diffusion in the cases mentioned may not however be an ordinary physical process, as we have evidence that in many cases, especially in endosperms, the cell-wall is perforated by very delicate strands of protoplasm. The most recent theory of the composition of

cell-wall also helps us to see a possible explanation of their passage, protoplasm or proteid being held to be present in its substance. That the protoplasm of the cell plays a part in permitting or preventing the diffusion we see from the two Torulas described, which retain their enzymes as long as they are living, though the latter can be extracted after killing the cells.

Reactions of the Enzymes.

But very few reactions can be quoted by which to attempt to identify these enzymes; indeed the manifestation of their activity is at present the only reliable evidence of their presence. From some observations Wiesner[1] made on the behaviour of diastase and pepsin, he gives as characteristic of ferments in general a colour-reaction obtained by heating them with an alcoholic solution of orcin in the presence of hydrochloric acid. Treated thus, diastase gives a bluish violet, and other enzymes give other colours. Further investigations into the behaviour of ferments with hydrochloric acid show that this reagent colours them differently. Thus Guignard[2], on boiling 1 centigramme of various ferments with 1 cc. of the pure acid, obtains the following effects: diastase yields a red turning brownish, emulsin a violet, papain an orange-red, trypsin a greenish-yellow.

These colour-reactions however prove not to be specially characteristic of the ferments. Reinitzer[3] has shown that dextrin, maltose, and lactose, all give similar colours, and other chemists have proved that the orcin-reaction is shared by nearly all carbohydrates, and is due to the production of furfurol. Udransky[4] has obtained it also from various proteids. Guignard, too, quotes the action of hydrochloric acid alone on various proteids, showing that these give colour-reactions much like those of the enzymes.

Relations of Enzymes and Organised Ferments.

To complete the discussion of ferment-action it is necessary to consider further the behaviour of the lower forms of plant-

[1] Sitzber. d. math.-naturw. Kl. d. k. Akad. d. Wiss. in Wien, July, 1885.
[2] Journal de Botanique, 1890, p. 393. [3] op. cit.
[4] Udransky, Zeitschr. f. phys. Chem. 1888 and 1889.

life which are capable of setting up complex decomposition in various substances, and which have been known therefore as organised ferments. Is there anything special about these that warrants their being still ranked as a separate class? They include a number of the lower fungi, the yeasts, and the great class of so-called micro-organisms or Schizophytes. The fermentations they set up are very varied, including the formation of alcohol from sugar, of various forms of acids from carbohydrate bodies, and the numerous products of putrefaction. Nägeli [1], in his theory of fermentation published in 1879, advanced reasons for considering them essentially different in their action from the enzymes considered in this paper, laying great stress on two points; (1) that they had not yielded to any extracting medium any thing that could effect fermentation in the absence of the cells, and (2) that the products of their action are 'without exception less nutritious compounds,' and that they destroy the most nutritious substances.

We must remember in considering their action that the micro-organisms are for the most part unicellular plants, and that therefore the whole round of their metabolic processes takes place in the same cell: the division of labour that can take place in a more differentiated structure is here impossible. Krukenberg [2] has shown that in the simplest forms the process of digestion is an intracellular one, not dependent on enzymes, but inherent in the protoplasm itself. Even in the higher forms we find a great many instances of this power of the protoplasm to effect chemical changes in the bodies with which it is supplied. In the ordinary metabolic processes of the vegetable cell we find it is the active agent, the chemical changes taking place not in the vacuoles but in the meshes of the protoplasmic network. Probably these are brought about by repeated combinations and decompositions, in which its own substance takes a leading part. We have evidence of processes of oxidation and reduction taking place there, leading to the appearance of various bodies ultimately be-

[1] Theorie der Gährung, München, 1879.

[2] Krukenberg, Vergleichend-physiologische Vorträge, Heidelberg.

coming simpler and leading to the production of CO_2 which always accompanies the vital processes. In the case of the alcoholic fermentation set up by yeast, Nägeli suggests that the living substance of the organised yeast-cell is to be regarded as being in continuous and rapid molecular vibration, and the decomposition of the fermentable substance is the result of the direct transference of these vibrations to the sugar, by means of which its equilibrium is upset and it is split into simpler and therefore more stable compounds[1]. But so great an authority as Pasteur regards alcoholic fermentation as indissolubly connected with the vegetative growth, multiplication, and metabolism of the yeast-cells. Sugar is so only the food-stuff out of which the organism obtains the material requisite for its metabolism and growth, the products of the fermentation being thus as it were the excretionary residues of the metabolised food. This is probable also from the fact that for the alcoholic fermentation to proceed, compounds of nitrogen must be supplied to the yeast as well as carbohydrate, and that the products of fermentation are not simply alcohol and CO_2, but that a certain amount of glycerine and succinic acid are also formed. What evidence do we find of similar action taking place in the higher plants? Lechartier and Bellamy[2] as well as Pasteur have shown that in certain ripe fruits alcoholic fermentation occurs. These exhale CO_2 in an atmosphere deprived of oxygen, sugar disappearing at the same time and alcohol being formed. The power of forming acids possessed by the fungus *Mycoderma aceti* and various bacteria is shared by the cells of succulent parenchyma. Though acetic acid is produced by the former plant from alcohol, and the parenchyma appears to form the acids it contains from sugar, the protoplasm in both cases seems to be the active agent. No enzyme can be extracted from the fruits alluded to which can form alcohol, any more than it can from yeast. The acids formed in the normal metabolism of the higher plants are not usually such simple ones as are originated by the microbes. We find malic, citric,

[1] loc. cit. [2] Comptes rendus, LXIX, 1869.

tartaric, &c., instead of acetic, lactic, butyric, &c. This may however be due to the character of the metabolism of the two classes of cells respectively, for the action is intracellular in both cases. Alteration of the conditions in which the cells are living may modify profoundly such metabolism, as we shall presently see. The power of the protoplasm to effect the disruption of carbohydrates is seen in the transformation and reconstruction of the transitory starch which is constantly going on in various parts of the higher plants, though no doubt in many cases here diastase takes part. The same power can be noted in the changes found to take place among the various sugars that the higher plants contain. Brown and Morris[1] have shown not only that cane-sugar is transformed into glucose and laevulose, but that it is also reconstructed during the growth of the embryo when germination has begun. They say that cane-sugar can always be detected in the embryo when artificially nourished on various culture-solutions, though not a trace of it be supplied in the culture-medium itself.

From a consideration of these phenomena it seems difficult to resist the conclusion that in both higher and lower forms we have to deal with what has been called the fermentative power of the protoplasm, and that the results that we note in connection with the working of the so-called organised ferments are only the expression of this activity, or in other words of the varying metabolism of the cells. The lower forms do not differ from the higher ones in possessing special powers, but only in not being able, from their want of differentiation, to show the division of labour which is so advantageous, if not necessary, to the latter. All the metabolic processes must be carried out in the unicellular organism in the same mass of protoplasm.

Returning to Nägeli's two points of difference between the action of organised and unorganised ferments, we have seen that the first of them can no longer be supported. From bacteria enzymes of several descriptions have been isolated;

[1] op. cit. p. 517.

from yeast and at least two of the fungi, *Fusarum* and *Aspergillus*, invertase has been extracted; from *Aethalium* a proteo-hydrolytic ferment has been obtained; from more than one fungus a cytohydrolyst has been prepared. Though the alcoholic ferment has not been extracted from the yeast-plant, this proves to be no peculiar property of that plant, as it cannot be isolated either from the fruits in which the formation of alcohol occasionally occurs.

Nor is there the radical difference in the nature of the products formed which Nägeli maintained. Boehm[1] and De Luca[2] have shown that if any part of a living plant he insufficiently supplied with oxygen, hydrogen, and sometimes marsh-gas are evolved from it. Boussingault[3] and Schulz[4] have observed similiar phenomena. From plants containing mannite also hydrogen is given off, while according to De Luca[5] acetic acid is formed from malic acid in the fruits, flowers, and leaves of the Privet. In the decomposition of proteid too Boehm[1] found ammonia exhaled. The condition under which these results are obtained, viz. the lack of oxygen, is the normal condition of many of the microbes, they being anaerobiotic in their mode of life. When oxygen is present we find the same agreement. The result of the action of invertase is the same, whether that action be brought about by living yeast, or by invertase extracted from a higher plant. The decomposition set up by trypsin, in the formation of albumoses, peptones, and amide-bodies, is similar to that induced by some of the proteo-hydrolytic bacteria. Sachs claims as a peculiarity of all fermentation set up by fungi that CO_2 appears as a bye-product[6]. This however we have seen to be rather an effect brought about by an insufficient supply of oxygen, and easily made evident under the same condition in the fermentative actions of the protoplasm of the higher plants also. We can see therefore that in both lower and

[1] Boehm, Sitzgber. d. k. Akad. d. Wiss. in Wien, LXXI, 1875.
[2] De Luca, Ann. d. Sc. Nat. Sér. 6, VI, 1878.
[3] Boussingault, Agronomie, t. iii, 1864.
[4] Schulz, Journ. f. Prakt. Chem. LXXXVII, 1862. [5] loc. cit.
[6] Sachs, Physiology of Plants, Engl. transl., 1887, p. 349.

higher plants we have to recognize essentially the same constitution, the differences between them only depending on differentiation, and consequent division of labour. In the lowly forms the great prominence of their metabolic decompositions has obscured all their other functions, and they have been therefore regarded as possessing special properties. In the higher plants investigation has shown us that precisely similar decompositions can be brought about, not now by the whole plant-body, but by special cells or parts of it. The agent in the decomposition is the same, the conditions similar, and the resulting products are strictly comparable. Instead therefore of speaking of organised and unorganised ferments, we come to recognize in the effects of them both only the power of the living substance to effect chemical change. In its most primitive form this is always intracellular, and involves the actual taking part in the decomposition by the protoplasm itself. Just however as in the slow movements of amoeboid protoplasm we recognize something which in the higher and more differentiated organism appears as the contraction of muscular fibre, so in this interaction we see a property which becomes more highly differentiated in the formation of enzymes, which work sometimes within and sometimes without the cells in which they are produced, the latter being the most specialised. The reason for the production of these enzymes is not always evident; in many cases it has been seen to be needful to induce decompositions at some distance from the seat of their formation, as when the embryo secretes them to acquire the contents of the endosperm in which it is embedded; in other cases, as in various microbes, it may well be to enable the plant, as Wood has suggested, to protect itself from adverse influence by forming a more resistant cell-wall; in many cases of intracellular enzyme-action, however, the present state of our knowledge leaves the matter unsolved.

NOTES.

ON THE REDUCTION OF THE CHROMOSOMES IN THE NUCLEI OF PLANTS.—Since the classical researches of Van Beneden on the development of the sexual cells and on the process of fertilisation in *Ascaris megalocephala*, which date from the year 1883, few points have been more studied than the reduction of the chromosomes (chromatin-segments), which is an essential feature in the development of the sexual cells, with the ultimate object of arriving at the physiological significance of this curious and interesting phenomenon.

Although the researches into this matter which have been made by botanists have hardly met with the attention which they deserve, it seems that it is by the study of these phenomena in plants that the next advances toward the solution of the problem are to be made. Already in the year following Van Beneden's first publication, it was shown by Guignard[1] and by Strasburger[2] that a reduction in the number of the chromosomes takes place in connection with the development of the reproductive cells of Angiosperms. Taking first the development of the asexual reproductive cells, the microspores (pollen-grains) and the macrospores (embryo-sacs) of these plants, it is conclusively proved that here a reduction in the number of the nuclear chromosomes takes place. Guignard[3] has ascertained that the nuclei of the mother-cells of the pollen in *Lilium Martagon* each contain only twelve chromosomes, whilst the nuclei of their immediate archesporial ancestors each contain twenty-four; and that similarly, the nuclei of the corresponding cells in species of *Allium* contain re-

[1] L. Guignard, Nouvelles Recherches sur le Noyau Cellulaire, Ann. d. sci. nat., Bot., sér. 6, t. XX.

[2] E. Strasburger, Neue Untersuchungen ueber den Befruchtungsvorgang bei den Phanerogamen.

[3] Comptes Rendus, t. CXII, 1891, p. 1074-76; also Nouvelles Études sur la Fécondation, Ann. d. sci. nat., Bot., sér. 7, t. XIV.

spectively sixteen and eight chromosomes. I have myself arrived at this result for *Lilium Martagon* independently of Guignard, as also for the following plants:—

	No. of chromosomes in Mother-cells of Pollen.	in Archesporial cells.
Scilla non-scripta and other species	8	16
Leucojum vernum	12	24
Triticum vulgare	8	16
Paeonia, several species	12	24
Aconitum Napellus	12	at least 20 probably 24

Again, it was established by Guignard[1] and by the writer[2] simultaneously that, in the Lilies and other plants where the cells which should correspond to the archesporium, to the mother-cells of the spores, and to the spores themselves of the higher Pteridophyta, are all represented by one and the same cell, the embryo-sac, the reduction in the number of the chromosomes occurs in the nucleus of the young embryo-sac. Strasburger[3] had, however, already pointed out that in other plants, such as the Orchids and *Allium*, where the mode of development of the embryo-sac is more primitive, the reduction takes place in the nucleus of the mother-cell of the embryo-sac; in a cell, that is, which is the morphological equivalent of a pollen-mother-cell.

Turning now to the sexual reproductive cells of the Angiosperms, it has been shown by Guignard[4] and the writer[5] that the number of chromosomes in the nucleus of the oosphere of the Lilies is the same as that in the nucleus of the embryo-sac, that is twelve. Guignard also showed that each of the generative nuclei in the pollen-tubes of the Lilies contains only twelve chromosomes. Strasburger[6] arrived at similar results for *Allium*.

From the foregoing facts it might fairly be concluded that, in the Angiosperms at least, the reproductive cells, whether sexual or asexual, contain only half as many chromosomes in their nuclei as do the

[1] Comptes Rendus, loc. cit. and Nouvelles Études.

[2] Beitrag zur Kenntniss der Entwicklung und Vereinigung der Geschlechtsproducte bei *Lilium Martagon*, Zürich, 1891.

[3] Ueber Kern und Zelltheilung, 1888.

[4] Nouvelles Études.

[5] loc. cit. [6] loc. cit.

vegetative cells; and the further inference might be drawn that the essential difference between vegetative (or somatic) and reproductive cells is indicated by the smaller number of chromosomes in the nuclei of the latter. But, though this course of reasoning may seem obvious enough, it is a question if it be the one which is really indicated by the facts. It must be borne in mind that the spore is the first stage of the sexual generation (gametophyte): hence when, as in the case of the Lily mentioned above, the same reduced number of chromosomes is to be found in both the embryo-sac (macrospore) and the oosphere on the one hand, in the pollen-grain (microspore) and the generative cells of the pollen-tube on the other, all these cells belonging to the sexual generation, it seems to be suggested that the reduced number of chromosomes in the nucleus is a feature which is peculiar, not to the reproductive cells, but to the whole sexual generation (gametophyte). This view of the matter is supported by the fact that during the division of the progamous nucleus of the pollen-grain we likewise find the reduced number of chromosomes, as was established by the three authors already mentioned.

In order to test the value of this latter hypothesis, it is essential that plants should be examined in which a greater number of cell-generations should intervene between the spore and the sexual reproductive cells; plants, that is, in which the gametophyte has a more pronounced individuality than it has in the Angiosperms, where only three cell-generations intervene between the development of the embryo-sac and that of the oosphere.

Thus, in the Gymnosperms a whole complex of cells, the endosperm, is formed in the embryo-sac before the female organs make their appearance. If now these cells show the same reduction in the number of the chromosomes in their nuclei as do the embryo-sac on the one hand, and the oosphere on the other, this reduced number will be proved to be a characteristic, not of reproductive cells only, but of the whole sexual generation.

For the investigation of this point *Ceratozamia mexicana* offers most suitable material, the nuclei being large and the number of chromosomes small. In the various parts of the asexual generation (sporophyte), in the nuclei of the young leaf, in those of the nucellus and its integument, the number of chromosomes is sixteen. In the developing gametophyte, the young endosperm, on the other hand, each nucleus contains but eight chromosomes, and this is the case long before the

formation of the archegonia, at a stage when no cells have as yet been formed within the embryo-sac, but only free nuclei imbedded in common protoplasm[1].

Although all the other Gymnosperms examined proved to be less favourable than *Ceratozamia* for the study of the karyokinesis in the embryo-sac, it was nevertheless ascertained that in *Tsuga canadensis*, *Larix decidua*, and in *Ephedra helvetica* the reduction occurs in the earliest stages of the development of the endosperm, whereas the nuclei of the cells of the nucellus and of the integument have the full number of chromosomes. It is therefore in the highest degree probable that the reduction in the number of chromosomes is effected during the formation of the embryo-sac, and persists through the whole female gametophyte (endosperm), including the oosphere.

The writer has also found that in the Gymnosperms, as in the Angiosperms, a similar reduction takes place in the mother-cells of the pollen and persists through the whole male gametophyte.

We are thus brought face to face with the fact that, so far as investigation has been carried at present, the sexual and the asexual generations in the Gymnosperms differ in that the nuclei of the latter contain twice as many chromosomes as do those of the former. It might even be possible to extend this generalisation so as to include the Angiosperms, were it not that Guignard[2] has observed that, in the Lilies, the antipodal nucleus possesses a greater number of chromosomes than does the sister-nucleus at the micropylar end of the embryo-sac; a greater number, in fact, than the primary nucleus of the embryo-sac contains[3].

Passing now to the higher Cryptogams (Pteridophyta and Mus-

[1] As the discovery of the attractive spheres (centrospheres) in plants is very recent, it may be mentioned that the young endosperm of *Ceratozamia* is a most favourable object for studying them. The writer has always been able to find them here without much difficulty. He has also seen them in *Taxus*, *Larix*, and several other Gymnosperms, also in *Leucojum*, *Paeonia*, *Aconitum*, &c.

[2] Guignard, Nouvelles Études, loc. cit. pp. 188 and 255.

[3] It will be well to bear in mind that the antipodal cells of the Lilies are of a very transitory nature, degenerating almost as soon as formed, and that irregularities in rudimentary organs are not uncommon. As a matter of fact the number of chromosomes in the antipodal cells of the Lilies, although it would appear to be always greater than twelve, is not constant, varying between sixteen and twenty-four. The writer thinks it not improbable that in many other Angiosperms the antipodal cells will be found to contain the reduced number of chromosomes. Further researches alone can settle this question.

cineae), where the number of cell-generations in the gametophyte is, generally speaking, much greater than in the Phanerogams, the question arises,—Does the reduction in the number of nuclear chromosomes take place as in the Phanerogams, and if so, does it take place at the same stage, or has there been a shifting of the morphological point at which this process occurs in the phylogeny of the vegetable kingdom?[1] In reply to this question I could say that the details of the karyokinesis in the spore-mother-cells of these plants correspond exactly to those seen in the mother-cells of the pollen. There is the same protraction of the first phases of division, the same thick and excessively short chromosomes, and the same early longitudinal division of the chromosomes. It will perhaps be asked why the number of chromosomes in the two generations was not directly ascertained. The answer is that in most of the Muscineae the nuclei are so small that the study of the karyokinesis is very difficult; whereas in the Pteridophyta, on the other hand, though the nuclei are large enough, the number of chromosomes in the forms hitherto examined is so great as to make even an approximate determination often almost impossible. At the same time, as far as a rough estimate can be of any value, such results as were obtained are favourable to the hypothesis that the reduction takes place in the spore-mother-cells, and persists throughout the gametophyte. I hope, however, to bring this question to a definite solution, either by finding more suitable material or by improving the methods of research. It will be a matter of great morphological as well as physiological interest, to establish beyond the possibility of a doubt that the alternation of generations, which is so remarkable a feature in the life-history of plants, is dependent on a change in the configuration of the idioplasm; a change, the outward and visible sign of which is the difference in the number of the nuclear chromosomes in the two generations.

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BOTANICAL NOTES, No. 4.—ON THE VELAMEN OF ORCHIDS.—It is a general rule that the roots of terrestrial orchids are devoid of a velamen, whilst those of epiphytic members of the

[1] This question was first raised by the writer, at the end of a paper read in January 1892 before the Zürich Botanical Society.

same class possess one. Hence it has usually been assumed that aerial orchids acquired their velamen subsequently to their adoption of the epiphytic mode of life. But Schimper[1], in his brilliant work on the epiphytes of South America, has pointed out that there exist epiphytic orchids which have no velamen, and terrestrial orchids which possess one. Hence it is quite possible that the terrestrial ancestors of an epiphytic orchid possessed a velamen. Schimper only discovered one epiphytic orchid which has no velamen; it is a species of *Stenoptera*, which grows in shady places on mossy or deeply furrowed bark. He found one purely terrestrial plant, *Epidendrum cinnabarinum*, which has a velamen. It may be pointed out that, with the exception of Schimper's species, *Stenoptera* is a purely terrestrial genus, and the genera closely allied to it are likewise terrestrial. Hence it is more than probable that Schimper's *Stenoptera* has only recently assumed its epiphytic mode of life; and this becomes all the more likely when its habitat is considered. *Epidendrum*, on the other hand, is typically an epiphytic genus, and its close relations are all epiphytic. So it is *a priori* probable that *E. cinnabarinum* has changed from an epiphytic mode of life to a terrestrial one; and in corroboration of this view may be mentioned the occurrence of curious tufts of aerial roots, concerning the physiological significance of which Schimper could frame no suggestion. These aerial roots are probably relics of the epiphytic stage of existence of the plant, and are a sign of an extremely recent change to a terrestrial mode of life.

It naturally suggested itself that light might be thrown on the question as to the date of origin of the velamen, by observations made, first, on orchids which naturally grow both as epiphytic and as terrestrial plants; secondly, on orchidaceous genera possessing both epiphytic and terrestrial species. During my stay in Singapore I had the inestimable advantage of Mr. H. N. Ridley's advice as to the selection of suitable plants, and he suggested *Grammatophyllum speciosum* and species of *Bromheadia* for the purpose.

Grammatophyllum speciosum.

According to Ridley there are few orchids of which individual plants grow naturally both as terrestrial and as epiphytic plants: but of these few, *Grammatophyllum speciosum* is one. This plant can be found growing on the ground in the jungle when it has happened to fall off a tree—it is

[1] Schimper, Die epiphytische Vegetation Amerikas. Jena, 1888.

an epiphyte which can grow as a terrestrial plant. In the Botanic Garden at Singapore a magnificent specimen of this gigantic orchid is growing planted in the soil. Its numerous stalks rise from the ground, and are densely beset with leaves. On approaching the plant one sees, under its shade, what resembles a low mass of dry whitened coarse grass. Closer inspection shows that this grass-like mass is really made up of numerous slender branched roots, which rise up out of the ground. Most of these roots have stopped growing and are white and stiff, but a few younger ones are less rigid, contain chlorophyll, and are light green in colour. Each of them takes origin from a thicker root which runs horizontally over, or beneath, the surface of the soil; it ascends obliquely outwards (towards the light) or is quite erect, but its youngest portion is always vertical. At its base it gives off secondary branches in various directions, but towards its apex the secondary branches dwindle and become arranged in one plane—the plane at right angles to the direction of incidence of the strongest light, so that on approaching the plants these pinnately-arranged secondary roots attract immediate attention. The diminution in the size of the secondary roots towards the apex is so considerable that they are mere tiny knobs, smaller, in fact, than a pin's head, even at some distance from the tip of the root. The larger basal secondary roots are directed towards the light, and give off tertiary branches, which are arranged in one plane just as are the smaller secondary roots. Each erect-growing root, as also its branches, only grows in length for a definite period, and then becomes hard and pointed. The bilateral nature of the portions of this root-system which are fully exposed to light, as denoted by the arrangement of the branches in one plane, recall Janczewski's[1] observations on the dorsi-ventral nature of many aerial orchid-roots. I have noted the same bilateral arrangement of the root-branches in the vertical roots of *Cymbidium aloifolium* growing in a pot; but usually the upright roots of pot-specimens of this plant are more or less devoid of branches.

But in the soil there is another system of roots quite different from that above described. These subterranean roots are thick and fleshy, and give off very few lateral roots. Immediately one of these roots ascends out of the soil it commences to branch, and in fact becomes one of the many aerial roots previously described. On the other hand,

[1] E. de Janczewski, Organisation dorsiventrale dans les Racines des Orchidées, Ann. d. sci. nat., Bot., 1885.

when an aerial root gives off a secondary root which dips back into the soil, the latter at once becomes thicker, but does not form tertiary roots, as do the secondary roots arising near the same spot on the aerial root. Another peculiarity is that a developing aerial root is longer than a subterranean root of the same age. Thus the aerial roots, while in actual process of growth, increase in length at a greater pace and branch more copiously than subterranean roots; but the aerial roots are thinner and their growth only lasts for a definite period.

Histology of the Subterranean Roots.—These roots possess a well-developed velamen, consisting of about eleven layers of tracheides; as also an epidermoidal layer (or exodermis), and about seventeen layers of cortical cells. The cortical cells, with the exception of the exodermis and one or two external layers of small cells, are structurally remarkable. Their walls have coarse broad reticulate thickenings which are lignified; the thin portions of the wall consist of pure cellulose and have small pits in them. Here and there in the cortex are large idioblasts, elongated in the direction of the long axis of the root, and possessing thick suberised walls; in their youth these cells contain raphides, which are subsequently dissolved.

In the vascular cylinder there are about fifteen radial series of large wood-vessels separated from the strands of phloëm by thick-walled parenchyma.

Histology of the Aerial Roots.—An aerial root, having a central cylinder not quite as large as that of the subterranean root described, has the following structure. Externally is an epidermal layer which is only one cell in thickness, excepting here and there where a cell is divided into an outer and an inner half. Each cell is flattened radially, but is elongated in the direction of the long axis of the root; its walls have delicate reticulate thickenings, which are directed at right angles to the long axis of the cell. The exodermis has thinner walls than in the subterranean root. The rest of the cortex is comprised of eight layers of cells, which are longer in the direction of the long axis of the root, but narrower than those of the subterranean root. In these cortical cells there is a tendency to form broad prominent bars of lignified membrane continuing from cell to cell, rather than to form the coarse irregular fenestrated thickenings seen in the underground root. The idioblasts are fewer in number and have thinner walls. In the vascular cylinder there are no radial lines of wide wood-vessels,

and no large sieve-tubes; but there is a relatively better developed pith than in the underground roots.

Descending the aerial root towards the ground, all the intermediate stages between the two extremes described, occur. The change in the form of the cells of the velamen, and their gradual increase in number, is especially noticeable.

Comparison of the Structure of the Aerial and Subterranean Roots.—The aerial roots exhibit a return to a simpler state. They are not roots which have merely stopped in development. The apex of an underground root shows a thick velamen forming at once close to the very tip of the root, whereas the apex of a root just commencing its aerial career shows already a diminution in its velamen; and the apex of an aerial root just about to stop growth altogether is practically quite similar to that of the root of any ordinary terrestrial plant[1]. It is still more remarkable that, when an aerial root dips down into the ground, it changes the character of its apex once more, and commences to form a thick velamen; such a root has a thin basal part, with only a few layers of velamen-cells, which are not markedly elongated at right angles to the axis; but nearer the apex the root is thicker and has a typical large velamen. Altogether it appears that, for some reason, the velamen is not fully developed unless the root be under the soil; whether this peculiarity is occasioned by the action of light or the distribution of moisture must be left undecided till direct experiments be made.

Conclusions.—Schimper has clearly explained the use of the negatively geotropic roots possessed by epiphytic orchids, and there is no reason to believe that they have any other function when they are developed in individual plants growing in the ground: that they act, for instance, as respiratory organs. The negatively geotropic roots are a sign of an epiphytic mode of life; and their occurrence in specimens of *G. speciosum* growing in the ground shows clearly that this plant is a typical epiphyte which can, however, grow as a terrestrial plant—and not the converse. This evidence is strengthened by the fact that *Grammatophyllum* is essentially an epiphytic genus. Hence, in *G. speciosum* we have an epiphyte which can be found growing wild as a terrestrial plant[2]. In this latter position, the velamen is not only

[1] Excepting the fine reticulate thickening of the one-layered velamen.

[2] Mr. Ridley tells me that *Cymbidium aloifolium* is not to be found growing as a ground-orchid, although it can be cultivated as such.

retained, but is evenly strongly developed under the soil: nor does the plant lose its epiphytic habit of giving off negatively geotropic roots. This case proves how readily an orchid, like *Epidendrum cinnabarinum*, could have given up its former epiphytic habits, without at the same time losing its velamen, or even parting with the tufts of aerial roots which it still bears.

Bromheadia [1].

The Malayan genus *Bromheadia* includes four species, of which two are epiphytic and two terrestrial. The epiphytic species are advanced representatives of their kind, in that they are lovers of intense light and grow at the tops of very tall trees (Dipterocarps), where many epiphytes would find existence impossible. Hence, any structural differences in the roots of the terrestrial and those of the epiphytic species, will throw more light on the effect of an aerial mode of life than would be the case if the epiphytic species lived in a semi-terrestrial position, such as in the moist, earth- and humus-filled pockets formed by the persistent leaf-sheaths of Palms.

Bromheadia alticola, Ridl. lives in the full blaze of the sun on high tree-tops. The plant from which I obtained my material grows on a tree-stump in the Botanic Gardens at Singapore. Its long aerial roots are brown in colour and feel rough when the hand is passed over them: they cling fast to the supporting tree. The root is enveloped with a velamen consisting of two layers of cells. The cells of the outer layer have highly suberised walls, and their inner walls are immensely thickened and traversed by pore-canals. But some of the outer cells grow out into root-hairs which, in regions not in contact with the support, are short, thickly cuticularised papillae. On the ventral side (i. e. in contact with the supporting tree) the hairs are long; of these hairs those nearest the dorsal side are long, thick-walled, and highly suberised, so that they only function as organs for fixing the root to the substratum; the rest of the ventral root-hairs have thin, slightly cuticularised walls, and flattened or lobed ends: according as the root is nearer to, or farther from, the actual surface of the substratum, the hairs are longer or shorter. The basal walls of the hairs are copiously pitted, obviously in order to permit the inward passage of liquids. Fungal hyphae permeate the outer layer

[1] H. N. Ridley, The genus *Bromheadia*, Linn. Soc. Journ. XXVIII, 1891.

of the velamen, but descend no deeper into the tissue of the root. The cells composing the inner layer of the velamen are elongated in the direction of the long axis of the root, and possess thin walls which show very delicate reticulate thickenings. The inner half of each end appears as if filled with a 'loosely coherent black-brown substance[1],' which is especially conspicuous outside the passage-cells, because the latter are more deeply placed than the other exodermal cells.

Beneath the velamen lies the suberised epidermoidal layer, or exodermis, which is made of small passage-cells, rich in protoplasm, and of elongated cells with only a thin film of protoplasm in each. Even the outer wall of a passage-cell has a thin, external, suberised layer, within which is a thick layer of cellulose. The rest of the cortical cells, and the vascular cylinder, call for no special description at present. The cortical cells are smaller in the ventral half than in the dorsal half of the root: so the vascular cylinder comes to lie in an excentric position, being much nearer the ventral surface. When a root is in close contact with the substratum its dorsi-ventral structure is very pronounced: its ventral surface is flattened: it has thin-walled, ventral root-hairs: the outer layer of the velamen possesses thinner walls than elsewhere: the cortical cells on the same side are smaller, and the vascular cylinder is excentric in position. When the contact with the support is not so close the flattening of the ventral surface does not take place. Finally, when the root is not in contact with any support it is radial in structure. Thus the dorsi-ventral structure is not induced by the action of light: contact and moisture come into play in initiating these structural changes. This last fact renders it at any rate possible that the dorsi-ventral structure in many orchid-roots is not solely a result of one-sided illumination, as Janczewski would have us believe.

It is worthy of note that, in structure and mode of action, this two-layered velamen differs as radically from a velamen constituted of tracheides, as it does from the ordinary epidermis of a root.

[1] This curious substance found in the innermost layer of the velamen of many epiphytic orchids, deserves more minute investigation. Thin sections show that it is made up of a mass of fine interlacing filaments forming a sponge-like mass. In the young cells these filaments are mainly arranged at right-angles to the inner walls of these cells of the velamen and are closely connected therewith. Possibly, in nature and mode of origin, this substance is somewhat similar to the intercellular rods formed in Marattiaceae. It turns yellow with all iodine-reagents, refuses to stain with Hofmann's blue, and forms in the cell very early.

The outer layer, excepting where root-hairs occur, functions as a sheath to prevent loss of water; the inner layer stores up water, absorbed by the root-hairs, and passes it on to the passage-cells of the exodermis.

Bromheadia palustris, Lindl. is a terrestrial orchid which lives in hot, sunny places, and is rarely found in shady or damp spots. The velamen is precisely similar to that of the preceding species, excepting that it peels off at once, and its hairs are reduced to thickly cuticularised papillae which scarcely protrude beyond the other cells. Thus there are never any normal root-hairs, acting as absorptive organs. Over any definite part of the root, the velamen lasts but little longer than the cells of the root-cap. Hence the epidermoidal layer is, almost at once, the external layer of the root. For the rest, the structure of the root is extremely similar to that of *B. alticola*. But compared with the latter plant the following differences may be noted. The cortical cells are longer and have more extensive intercellular spaces between them; in addition there occur little groups of cells, similar to the cortical cells of *Grammatophyllum*, with lignified reticulate thickenings and thin pitted areolae of cellulose. In the vascular cylinder the bundles are more numerous, that is, there are more numerous radial series of wood-vessels alternating with masses of phloëm; but the most striking distinction consists in the larger pith which is made up of more numerous and larger cells, and has a more considerable intercellular system.

I noted some roots slightly flattened on one side: the vascular cylinder was approximated to the same side, and the cortical cells were smaller than on the reverse half. Probably this lack of radial symmetry was merely caused by the root coming in contact with a stone or some hard object.

In the cortical cells mycorhiza is present. It does not penetrate into the vascular cylinder, nor is it found in the elongated cells of the exodermis; but often a passage-cell contains very thick, yellow, glistening, mycorhizal hyphae, which can be traced into adjoining cells as fine hyphae, and often appear continuous with still finer hyphae outside the root. The mycorhiza is not found in the part of the root over which the velamen is still present. It is quite possible that this is due to the fact that hyphae cannot penetrate the thick outer layer of the velamen, in which case the absence of endotrophic mycorhiza in *B. alticola* is easily explained.

Conclusions drawn from Observations on Bromheadia.—Ridley is of opinion that *B. palustris* has descended from epiphytic ancestors. The structure of its root harmonises with this view; its velamen is fundamentally similar to that of the epiphytic *Bromheadia*, but it lasts for a short time only. It is easy to perceive the use of the thick-walled outer layer in the case of the epiphytic form which grows in the full blaze of the sun. But with regard to the terrestrial *B. palustris*, the most rational explanation of the precocious development and speedy dissolution of this thick-walled layer with its blunt dwarf-papillae, is to suppose that the curious velamen is a relic handed down from an epiphytic ancestor. But, however this may be, the persistence of the velamen in the epiphyte and its early disintegration in the ground-orchid, together form a strong piece of corroborative evidence that the velamen is essentially adapted to epiphytic plants, and was evolved in each case subsequently to the assumption of an aerial mode of life by the plant. The occurrence of the velamen in two such widely separated families as the Orchidaceae and the Aroideae, further strengthens this view. And, finally, the well-known fact that an increase in the intensity of the sunlight to which any leaf or green branch is habitually exposed induces an elongation of the epidermal cells in a direction at right angles to the free surface, and even to their division into outer and inner halves, shows that in the formation of the velamen there is nothing exceptional or unexpected.

General Conclusions.—The foregoing observations on *Grammatophyllum* and on *Bromheadia* seem to lead to contradictory conclusions. For whilst, in the former, the velamen is more highly developed in the subterranean root, in the latter it is more highly developed in the aerial root. The solution of this apparent paradox is afforded by the consideration that the function of the velamen is not the same in all cases. Thus, in *Grammatophyllum* the velamen is essentially an absorbent organ, which not only persists, but assumes a higher development, when the root is subterranean: whereas in *Bromheadia* the velamen is mainly protective, preventing loss of water by transpiration, the absorptive function being carried on by the root-hairs on the ventral surface of the root. Hence, in the aerial branches of the subterranean roots of *Grammatophyllum* the velamen dwindles, whilst in the subterranean roots of *Bromheadia* it peels off so as not to interfere with the process of absorption.

BOTANICAL NOTES, No. 5.—THE INFLUENCE OF EXTERNAL CONDITIONS ON THE FORM OF LEAVES.—When at Singapore, I requested Mr. Ridley to show me some examples of plants growing in the Botanic Gardens in a habitat not usual to them. In response, Mr. Ridley was good enough to point out an undescribed species of *Renanthera*, to which he proposes to give the name of *R. albescens*, an epiphytic orchid which naturally scrambles over plants growing on hot open sandy heaths. The specimen had been transferred to the Botanic Garden, where it was growing under the shade of a well-foliaged tree. The interest in the specimen lies in the fact that *on one and the same plant* it was possible to see the effect of environment on the form of the shoot. The part of the shoot which had developed when on the sandy heath consisted of a stiff stem bearing short thick leaves separated by short internodes, whilst the part of the shoot developed in the garden had longer and thinner leaves, which were separated by longer internodes. A table will best represent the comparative structure of the two sets of leaves.

Leaf formed on Sandy Heath.		*Leaf formed in the Gardens.*
60 mm.	Length.	120 mm.
28 mm.	Maximum width.	25·5 mm.
1·75 mm.	Thickness.	·925 mm.
	STRUCTURE.	
Thick cuticle.	Upper epidermis, with no stomata.	Cuticle half as thick, cells shallower.
1 layer of flattened cells. 1 layer of 'polygonal' cells. 12 layers of large parenchyma-cells, elongated at right angles to the surface. 2 layers of small polygonal cells.	Mesophyll, of about the same number of layers of cells in both leaves; no distinct pallisade-cells or spongy parenchyma; intercellular spaces small; scattered prosenchymatous cells, with thick cellulose-walls.	Several layers of flattened cells, merging into large polygonal cells, not markedly elongated at right angles to the surface. 2 layers of small polygonal cells.
Thick cuticle. Many stomata level with the surface.	Lower epidermis.	Cuticle half as thick. Many stomata slightly raised above the surface.

Thus the decrease in thickness of the leaves grown in the gardens is occasioned by a change in the form of the cells, and not by a diminution in their number. The combined effect of strong sunlight

and drought acting on the leaves is the same as in terrestrial plants; there is a distinct elongation of all the cells in a direction at right angles to the surface; the cuticle is better developed, and the leaf as a whole is thicker and smaller.

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ON THE RESISTING VITALITY OF THE SPORES OF BACILLUS MEGATERIUM TO THE CONDITION OF DRYNESS. During the autumn of 1885 and spring of 1886 I was engaged with bacterial studies in the laboratory of my friend Professor M. Hartog of Queen's College, Cork. He had brought from Strasburg the dry spores of a cover-glass culture of *B. Megaterium*, prepared by Professor De Bary in his laboratory on August 1884. From this, in the early spring of 1886, we succeeded in raising pure cultures with nutrient gelatine, and we afterwards kept the *Bacillus* under observation in good condition for several months. The usual method of cultivation practised by us was that of the hanging-drop, on cover-glasses, inoculated from gelatine-colonies, and then inverted on a damp filter-paper-cell which was placed on an ordinary slip, and kept at the temperature desired in a damp chamber; we found the *Bacillus* to develop favourably in a solution of filtered milk and water with a little added glucose; or in a 50 % glucose solution with a trace of Liebig's extract. Many of our cultures so prepared were carefully dried off and distributed among the botanical and pathological laboratories of Great Britain and Ireland.

On March 1886 I prepared a special culture of *B. Megaterium* for public observation at a conversazione held in the Art Schools at Cork. A young pure cover-glass culture was chosen and transferred to a cell of millboard saturated with paraffin-wax, the edges of the cover-glass being luted on with warm paraffin-wax, with the object of keeping the cell air-tight. The cultivation was on view for three successive evenings, and appeared quite pure: the *Bacillus* showed characteristic motion, which lasted nearly two days, when it settled down, and gradual spore-formation became visible: on the third evening the spores were mature, and the thin film of liquid hanging on the cover-glass appeared to have in greater part evaporated.

I carefully preserved this cultivation among my other slides, and had occasion to examine it several times at long intervals, till November 1890, when, having offered to demonstrate a lecture on decompo-

sition for my friend Professor Letts of Queen's College, Belfast, it occurred to me to try and revive the spores of my old culture of *B. Megaterium* which had then been torpid for nearly five years. I had not sufficient time to attempt isolation with a gelatine-plate, so inoculated direct from the spores on the old cover-glass, after first soaking them for a short time in a drop of sterilised water. I used an ordinary platinum needle, and with the usual precautions mounted twenty cover-glass cultivations, also a few blind duplicate-cultures of the media employed. The batch was then removed to a situation where a temperature of about 80–85° F. was maintained. I first examined these cultivations after about twenty-four hours, when they all appeared sterile: on a second examination the following day, however, I found one single development, and as the *Bacilli* then appeared settling down for spore-formation (their motion having almost ceased), I am inclined to think that this single growth had escaped my observation the previous day. Nearly all the other mounted spots remained quite sterile for several days after.

The cultivation was not a pure one; but the contrast in size between *B. Megaterium* and *B. subtilis* (with which I think it was contaminated) was very marked.

This development of *B. Megaterium* from its spores seemed to me quite normal as regards time, and partly for this reason I think the observation an interesting one.

I am inclined to think that my want of success with the remaining nineteen cultures of the batch, may have been due to actual rupture of the cells in attempting to remove them with the platinum needle. But it may well be that the life-limit of the spores had been reached in the majority of cases; and that the successful culture was due to a variation in the direction of longevity. This would put the life-limit of the spores of *Bacillus Megaterium* at about four and a half years, which is far below that of *B. subtilis* or *B. Anthracis*.

I again tried to revive the remaining spores of the same old cultivation in December 1891: the cover-glass had been broken in several pieces, and had remained exposed for more than a year under an inverted tumbler in my laboratory at Bushmills. I used both solid and liquid media, but on this occasion failed with both—I, however, attach no absolute confidence to this negative result.

ALLAN P. SWAN, Bushmills, Co. Antrim.

On the Development of Azolla filiculoides, Lam.

BY

DOUGLAS HOUGHTON CAMPBELL,

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With Plates VII, VIII, and IX.

THE genus *Azolla*, though a small one, has representatives in all the divisions of the globe, except Europe, and here, according to Belajeff[1], one of the American species, *A. filiculoides*, has been introduced of late years. Of the four species given by Strasburger[2], two, *A. filiculoides* and *A. caroliniana*, are American; *A. pinnata* is found in Australasia, Asia, and Africa; and *A. nilotica* is exclusively African.

Of the American species, *A. filiculoides*, the subject of the present paper, is confined to the western part of America, being reported from as far south as Chile, and reaching to California at least, and probably beyond. Until very recently American botanists confounded this species with *A. caroliniana* of eastern America, and in the Botany of California[3] only that species is mentioned. I have examined material from various parts of California, and in all cases the plants were undoubted specimens of *A. filiculoides*. Whether further

[1] Ueber das männliche Prothallium der Rhizocarpeen; Botanisches Centralblatt, 1892, No. 24.

[2] Strasburger: Ueber *Azolla*, Jena, 1873.

[3] Geological Survey of California. Botany, vol. ii, p. 352.

[Annals of Botany, Vol. VII. No. XXVI. June, 1893.]

examination will show *A. caroliniana* to occur in California, remains to be seen, but its occurrence must at present be regarded as doubtful.

A. filiculoides is common in many parts of California, and often occurs in great quantity, so that the surface of a pond, or a quiet stretch of river, may be completely hidden. As the leaves are strongly tinged with purplish red, the plants are then very conspicuous and recognizable from some distance away.

Observations were begun in November, 1891, and continued, except during the summer, until October, 1892. Most of the material was taken from a pond about a dozen miles from Palo Alto, but specimens were also received from various points south. For some reason the plant disappeared completely from the pond mentioned during the past summer, and in September no trace of it was to be found except the fragments of the dead plants. As the plants were abundant and vigorous the year before at about the same time, it seems hardly likely that this is always the case. Ripe spores that germinated promptly were obtained from the decaying fragments of the plants, and probably will serve to stock the pond again, as the plants spread with great rapidity when once established.

Our knowledge of *Azolla* is based mainly upon the papers of Strasburger[1] and Berggren[2]. Several earlier writers, Griffith, Mettenius, Meyen, and Martius, are referred to by Strasburger, but their works were not accessible. The results of their observations, however, are given by Strasburger in his admirable monograph of the genus.

Strasburger's work deals very exhaustively with the anatomy and histology of the mature sporophyte, and also of the full-grown sporangium and spores. The development of the latter he was not able to follow for lack of material. Berggren's paper deals with the female prothallium and embryo, and while valuable as the only contribution to our knowledge of

[1] loc. cit.

[2] Om Azollas prothallium och embryo. (Lund's Univ. Arsskrift. t. xvi.)

the subject, is very incomplete, especially with reference to the early stages of the former. Belajeff[1] has published an account of the male prothallium of *A. filiculoides* in a recent paper on the male prothallium of the Rhizocarpeae.

The same methods were used by me in the study of *Azolla*, that I have found most successful in the study of other delicate plant-tissues. The material was fixed with a 1 per cent. aqueous solution of chromic acid, stained *in toto* with alum-cochineal, and then imbedded in paraffin. After sectioning, the sections were stained on the slide with Bismarck-brown in 70 per cent. alcohol. As yet I have found no other method which gives such good results.

Before considering the development of the sporangia and spores it may be well to describe briefly the structure of the mature sporophyte. As Strasburger has treated this very exhaustively in the memoir referred to, it will not be worth while to go much into detail here. The plant is strongly dorsi-ventral; the leaves form two alternating rows completely concealing the stem. Each leaf is deeply two-lobed, and in the dorsal lobe is a large cavity in which is always found a colony of a *Nostoc*-like plant, *Anabaena Azollae*.

The growing-point of the stem is curved upward and backward, so that longitudinal sections parallel to the surface are very difficult to get. The stem grows from a single apical cell (Pl. VII, Figs. 2–4, x), from which two series of segments are cut off with great regularity. Each segment now divides into a dorsal and ventral cell, so that a transverse section of the stem, just back of the apex, shows usually four cells arranged like quadrants of a circle (Fig. 5, I.). From the dorsal cells the leaves are developed: from the ventral, the lateral branches and roots. An examination of the growing-point of the stem shows that it is more or less surrounded by a tangle of *Anabaena*-filaments, and some of them creep into the cavities in the young leaves and form the beginning of the colony referred to.

The mother-cell of a leaf is distinguishable by its size and

[1] loc. cit.

position (Fig. 5, III. *L.*); and the first division-wall in it divides it into two nearly equal cells, which develop into the two lobes found in the full-grown leaf. No trace of an apical cell can be found even in the youngest leaves, and in this respect, as well as in the secondary divisions of the segments of the apical cell, *Azolla* differs from *Salvinia*, its nearest relative. Each leaf-lobe is next divided into an inner small cell, and an outer larger one, and the latter then is divided into two equal cells by a radial wall. This formation of alternate tangential and radial walls is repeated with great regularity in the ordinary leaf-lobes, and in their young stages can be traced for a long time.

The sporocarps or sori always arise, as Strasburger showed[1], from the ventral lobe of the first leaf of a branch. He was not able to get the earliest stages owing to lack of material, and based his conclusions upon a study of the later stages. According to his statement they arise from a part only of the ventral lobe, the rest giving rise to the enveloping involucre. From a careful study of the very young stages I have been led to a somewhat different conclusion. I find that the whole of the ventral lobe goes to form the sori, and that the involucre is derived from the whole of the dorsal lobe of the leaf. The latter, instead of developing as in the sterile leaves, remains but one cell thick, and forms a sort of hood arching over the sori. The latter are always found in pairs in *A. filiculoides*. These are sometimes of the same kind, or one may be male and the other female.

The leaf-lobe which is to develop into the sporocarps is distinguishable at an extremely early period. Its first divisions are like those in the sterile lobes, and like them it is divided into two very nearly equal parts. Each half now developes at once into a sporocarp. As soon as the first median wall is formed, each of the resulting cells becomes the initial cell of the future sporocarp. In it walls are formed that cut off three segments from its base, and these are followed by others following the same order, so that for some

[1] loc. cit. p. 52.

time each sporocarp-rudiment grows by a three-sided apical cell (Fig. 8). Next a slight outgrowth is observed near the base of the young sorus, which forms a ring-shaped projection around it (Fig. 9 *id.*); this is the beginning of the indusium or sporocarp-wall, and corresponds exactly to that of *Salvinia*[1].

From this point the two sorts of sporocarps differ. In the macrosporic ones the apical cell forms at once the body of the single sporangium; in the microsporic, it forms a columella, from which latter the microsporangia arise as lateral outgrowths. My own observations in these earlier stages were confined mainly to the female sorus, but there was nothing to indicate any difference in the development of the male, except in the sporangia themselves.

There has been more or less conjecture concerning the origin of the single macrosporangium, whether it was really the only one formed, or whether several were formed at first and one crowded out the others. A glance at a young sporocarp will show at once that only one sporangium is formed at first, and that this is formed directly from the apical cell of the sporocarp-rudiment. After a varying number of segments have been cut off, a periclinal wall is formed in the apical cell, which then proceeds at once to form the body of the sporangium. At this stage (Fig. 9) the sporangium has a very short pedicel, and the archesporium has the familiar tetrahedral form common to the other Leptosporangiatae. The next divisions follow closely those of the other Leptosporangiates, and offer nothing new. The tapetum (Fig. 10 *t*), is formed as usual, and, coincident with its formation, radial walls are formed in the outer cells of the sporangium, whose wall, as usual, remains but one cell thick. The tapetum also divides only by radial walls, so that it too consists of but one layer of cells (Figs. 12–15). These cells, as well as the central cell, contain more granular protoplasm than do the cells of the sporangium-wall.

The divisions in the central cell correspond with those in other Leptosporangiates. The first wall is nearly vertical, and this is followed by a transverse wall in each of the resulting

[1] Strasburger; Ueber *Azolla*, p. 54.

cells. Finally, in each of these four cells, another wall arises, so that eight cells in all are formed. While these divisions have been going on, numerous radial walls have arisen in both tapetum and sporangium-wall, the former being especially numerous.

Shortly after the divisions are completed in the central cell and tapetum, the cell-walls of the latter are dissolved. At first the group of spore-mother-cells remain together; but, finally, their walls are sufficiently dissolved to completely isolate the eight cells, which are then surrounded by the fused contents of the tapetal cells. Each of the eight spore-mother-cells, as usual, gives rise to four spores. In *Azolla* these are of the tetrahedral type.

At the time that the spore-mother-cells are about to separate, the nucleus is large, but contains but little chromatin and consequently does not stain deeply. The nucleolus is much less conspicuous than in the earlier stages, and after the cells are completely separated it becomes scarcely distinguishable.

The divisions of the nucleus can be traced without much trouble, but owing to the small amount of chromatin, and the correspondingly small size of the nuclear filaments, the karyokinetic figures are small, and the details difficult to follow. As there did not seem to be any deviation from the process as seen in the division of the spores of other plants, no special observations were made. The nuclear spindle is clearly defined, but small (Fig. 18 *b*); after the first nuclear division is completed, the daughter-nuclei divide again before any division in the protoplasm is to be seen. Then follows the simultaneous division of the protoplasm into the four young spores (Figs. 19, 20). Of the thirty-two young spores thus formed, only one comes to maturity, and the others are used up in its growth.

The spore which is to form the macrospore increases rapidly in size. It is at first a thin-walled oval cell, which lies free in the enlarged cavity of the sporangium. Examination shows that it is surrounded with a thick layer of densely granular protoplasm, in which may be plainly seen a number of nuclei,

evidently those of the tapetal cells (Fig. 21). It is evident at a glance that these nuclei are directly concerned in the growth of the spore, and that they play an important part in the formation of the extraordinary appendages of the ripe spore. Unfortunately, owing to my absence during the summer, I failed to get the later stages in the development of the macrospore, but to judge by analogy with the microspores, and also by what is known in the case of the Marsiliaceae, the outer spore-coverings are derived from the protoplasmic mass in which the macrospore is imbedded. This protoplasm is evidently active, as is shown by the presence of normal nuclei, as well as by its increase in bulk as the sporangium increases in size.

When the sporangium is about half grown, the outer cells of the very short stalk grow out into short papillae (Fig. 16 *sp.*), which apparently are abortive sporangia, as they show divisions which recall the earlier ones in the macrosporangium. Their position corresponds to that of the microsporangia, so that although formed much later than the macrosporangium, it is pretty safe to assume that *Azolla* is derived from some form in which, as in *Salvinia*, there were several macrosporangia in the sporocarp.

The indusium grows much faster than the sporangium and soon completely encloses it. It grows mainly by the activity of the marginal row of cells, in which divisions are cut off alternately upon the inner and outer sides. After reaching above the top of the sporangium the edges converge, and this, together with an enlargement of the marginal cells, finally completely closes the opening.

Before the opening closes, filaments of the *Anabaena* creep in and form a mass filling all the space between the top of the sporangium and the opening of the sporocarp. Here the cells separate completely, and the plant enters a resting-stage to resume its activity on the germination of the macrospore.

In studying the development of the macrosporangium, I could not but be struck by the extraordinary resemblance to that of an ovule. The close investment of the sporangium

by the indusium, and the origin of the latter from so near the base of the sporangium, at once suggest the possibility of a homology between the indusium and the first integument of the ovule. Prantl[1] has advanced the view that an ovule might be regarded as a monangic sorus, and the integument as homologous with the indusium. So far as I can find, *Azolla* has not been considered in the arguments for and against this view, but it certainly supports strongly the view held by Prantl[2]. Of course this does not imply a direct relationship between *Azolla* and the Spermaphytes, as the sporangia themselves are widely different, *Azolla* being typically lepto-sporangiate, while the Spermaphytes are eusporangiate. Nevertheless, in view of all the facts bearing on the matter, it hardly seems necessary to regard the ovular integument as an entirely new structure, without any equivalent among Pteridophytes.

The ripe female sporocarp is about 1·5 mm. × 1 mm. in size, and strongly pointed at the top. Its outer cells become hardened, and those of the upper half very dark-coloured and lignified, so that after the lower part decays, these upper, dark-coloured cells remain as a little cap that covers the spore until it is thrown aside by the growth of the embryo. The inner cells of the wall remain thin, and become very much compressed by the growth of the sporangium which finally fills the sporocarp completely. The wall of the sporangium does not, however, as stated by Strasburger[3], become absorbed. On the contrary, it can be plainly seen in sections of the full-grown sporocarp (see Pl. VIII, Fig. 38), but the cells are much flattened, and unless carefully examined might be taken for the inner layer of the indusium, which is compressed so as to be almost obliterated.

A longitudinal section of the full-grown sporocarp (Fig. 35) shows that the spore[4] with its curious appendages fills the

[1] Untersuchungen zur Morphologie der Gefässkryptogamen: Die Schizaeaceen, p. 154.

[2] Since the above was written, I find that Eichler has called attention to the resemblance between the sorus of *Azolla* and an ovule: see Engler and Prantl, Die nat. Pflanzenfam. II. Theil, 1. Abth. p. 16.

[3] loc. cit. p. 71.

[4] Sometimes two spores are found in one sporangium.

sporangium completely, and that the latter is in close contact with the indusium except at the top, where there is a space between filled with the resting cells of the *Anabaena* (*n*). The sporangium-wall at this point (Fig. 35 *sp.*) is perfectly plain, but the cells have collapsed so that the separate cells are not very easily distinguishable. Near the base of the sporangium, however, they may generally be very easily seen, especially when, as often happens in sectioning, the wall is pulled away from the indusium. (See Fig. 38 *sp.*).

The curious episporic appendages of the macrospore have been frequently described, but the homologies of the parts have not been entirely understood. The ripe spore is perfectly globular and surrounded by a firm yellowish exospore, which appears almost perfectly homogeneous in section. Upon this is deposited a thick epispore of most peculiar form. This is covered with cylindrical papillae from the top of which numerous curious threadlike filaments extend. In section the epispore shows two distinct portions, an inner mass resembling exactly the substance of the massulae, and a denser part that covers the outside except the tips of the papillae. This outer part is solid and nearly homogeneous, in places densely granular. The epispore covering the top of the spore is developed in a most extraordinary manner. It consists here of four parts; a central conical part, and three somewhat pear-shaped masses that are partly sunk in shallow cavities in the sides of the central portion. To these Strasburger applied the name 'Schwimmapparat,' supposing them to be filled with air, and to thus raise the spore to the surface of the water. Repeated experiments with perfectly ripe spores, both before and after they had been freed from the indusium, resulted invariably in the spores sinking immediately, as was the case with the ripe massulae. This being true, the name must be abandoned as misleading. From the conical mass, as well as from near the apex of the others, the filaments, like those growing from the papillae of the lower part of the spore, are produced in great numbers. In the spaces between the masses, even in the full-grown

sporangium, the remains of the tapetal nuclei (Fig. 36 *n*) may be seen occupying much the same position with reference to these that those of the microsporangium do to the massulae. This, together with the similarity in the structure of the epispore of the macrospore, and the massulae, warrants the conclusion that the two are homologous structures. The threads attached to the epispore may morphologically as well as physiologically be compared to the glochidia.

Sometimes, but not always, a cleft may be seen extending upward part way through the central conical mass, but in no cases is there such an open canal as described and figured by Berggren[1] for *A. caroliniana*.

The Microsporangia.

In the male sorus, as we have already seen, the apical cell of the young rudiment does not form a sporangium, but gives rise to a central columella or placenta, from which the microsporangia arise laterally, while the end projects as a cylindrical body (Fig. 22 *col.*). This latter was observed by Meyen[2], but Strasburger seems for some reason to have overlooked it. I found it in all my sections of the male sorus. As in the female sorus, the indusium is two cells in thickness, but the cells have their walls more uniformly thickened, and the inner layer is not compressed as in the female sorus; as in the latter, the opening at the top becomes completely closed, and the cells about it are thicker walled than the lower cells, and reddish brown, as in the upper cells of the indusium of the female sporocarp. Like that, too, the top is pointed, but the point is short and abrupt, and the body of the sporocarp is globular. They are more than twice as large as the female sporocarp. Both sorts of sporocarp have a very short stalk, into which a fibrovascular bundle extends for a short distance.

The development of the microsporangium corresponds closely to that of the macrosporangium, but differs in some

[1] loc. cit. p. 1; also, Fig. 1.

[2] See Strasburger, loc. cit. p. 57.

respects, especially in the later stages. While the macrosporangium has a very short massive pedicel, that of the microsporangium is long and slender, usually composed of two rows of cells, but not infrequently showing three. Up to the third division of the central cell of the sporangium the divisions are exactly as in the macrosporangium; but in the microsporangium there is one more division and consequently sixteen spore-mother-cells. The sixty-four spores that result from the division of these, all develop more or less completely, and about each is formed a smooth, thin, yellowish exospore. The ripe spore is about ·035mm. in diameter, and its apex shows plainly the usual three radiating lines. The full-grown microsporangium (Fig. 26) is globular, its walls formed of tabular cells all about alike. I was unable to detect anything that looked like the annulus of the homosporous leptosporangiate Ferns. The complete disappearance of the annulus is no doubt attributable to the aquatic habit of *Azolla*.

When the spores are nearly mature, the formation of the massulae or masses of hardened protoplasmic matter in which the ripe spores are imbedded, begins. The spores collect in several groups (usually about five), and about these the protoplasm lying between them becomes aggregated. Apparently vacuoles are formed in this giving it a foamy appearance, and finally these become so large as to give the massula the appearance of a cellular tissue (Figs. 24, 27, 28). In *Salvinia*, where there is no division into massulae, according to Strasburger's[1] account, the nuclei of the tapetal cells are scattered uniformly through the protoplasm lying between the spores; but in *Azolla*, they are confined to the outside of the massulae, where they can be readily detected almost up to the time of the ripening of the sporangium. As the massulae mature there are formed upon the outside the *glochidia*, curious hair-like appendages with anchor-like tips. They are formed in the spaces between the massulae, and their flattened form is due to the narrowness of these spaces. The presence of the unchanged tapetal nuclei about the forming *glochidia*

[1] Bau und Wachsthum der Zellhäute, p. 133.

(Fig. 24 *n*), indicates that, like the appendages of the macrospore, these too are formed in part, at least, by the activity of the tapetal nuclei. In *A. filiculoides* the glochidia are undivided, except occasionally toward the tip, where one or two septa may sometimes be detected.

When the sporocarp of *Azolla* is compared with that of the other Hydropterideae, its nearest approach is found in *Salvinia*, with which it agrees closely in its origin and structure. Each sporocarp is a single sorus with a cup-shaped, completely closed indusium, while in the Marsiliaceae the sporocarp represents a whole leaf-segment with several sori. Among the homosporous Ferns, some of the Cyatheaceae and Hymenophyllaceae (especially *Trichomanes*), show very marked resemblance to the Salviniaceae in the position and form of the indusium.

Germination of the Microspores.

The study of the germinating spores offers great difficulties, as they are completely imbedded in the massulae, and cannot be freed in the living state. Belajeff[1], who has recently published some observations upon the male prothallium of *A. filiculoides*, treated the massulae with chromic acid which rendered them brittle enough to be broken in fragments, thus setting free the prothallium. My own observations were made mostly from sections. As the massulae adhere to the macrospores, in making sections of the latter, the massulae, with their enclosed spores, were also sectioned. Of course in this way it is impossible to regulate the direction in which the sections are made, but enough straight sections were obtained to give a clear idea of the development of the antheridium. In the material used by me, only a small percentage of the microspores seemed perfectly developed, and in consequence, the number of male prothallia found was very small compared to the number of massulae sectioned. As the germinating spores are completely buried in the massula, it is very difficult to judge of the state of develop-

[1] loc. cit.

ment, and I was unable to find fresh specimens with ripe antheridia and so failed to see the free spermatozoids; otherwise my observations on the antheridium were fairly complete.

The indusium decays slowly and the sporangia are thus set free, after which the wall of the latter also decays, and the massulae escape into the water. Contrary to the statements usually made, it was found that the ripe sporangia, and also the massulae themselves, sink at once to the bottom when placed in clear water. When entangled in the remains of the plant, it is true that they float, but this is not due to their own buoyancy, but to the air in the tissues of the stem and leaves.

As soon as the sporangium-wall is decayed, the glochidia stand out straight from the surface of the massulae. As they come in contact with the macrospore, they fasten themselves to it by means of the anchor-like ends of the glochidia.

The contents of the microspores are not very dense, and they contain but little granular matter. The nearly central nucleus, which is not very rich in chromatin, shows a more or less conspicuous nucleolus. The first indication of germination is the rupturing of the exospore along three radiating lines at the top, and the protrusion of a papilla through this (Fig. 29). This papilla is then cut off by a wall near the top of the spore-cavity, and forms at once the mother-cell of the single antheridium. The next divisions were not satisfactorily made out. According to Belajeff[1], the next divisions are nearly parallel to the first and divide the antheridium into three cells, one above another, and of these only the middle one undergoes further division. For some reason which is not clear from his account, Belajeff does not regard the whole of the upper cell as an antheridium, but states that the latter is only formed after five vegetative cells are cut off. It seems much more in accordance with what obtains in the related homosporous forms to regard the whole of the upper part of the prothallium as an antheridium. In spite of his statement

[1] loc. cit. p. 329.

that the development of the male prothallium has little in common with that of the true Filices, the figure of the prothallium of *Azolla* given [1], bears a very striking resemblance to the simple male prothallium of many Polypodiaceae, for instance. The small cell, cut off subsequently from the large basal one, as described by him, I failed to see in any of my sections. No indication of marked dorsiventrality, as he states, was noticed either. This may possibly have been due to slight shrinkage in imbedding, by which the central part of the prothallium was a good deal more constricted than it probably is in life. My own conclusion, reached after a careful study of a large number of prothallia, is that there is but a single vegetative cell formed (from which possibly later a small cell may be cut off), and that the rest of the prothallium forms at once a single terminal antheridium.

The subsequent divisions, as observed by me, correspond essentially with those given by Belajeff. In the middle cell of the antheridium two nearly vertical walls are formed, and with the upper cell (Fig. 31 *o*) completely enclosing the central cell of the antheridium. The cell (*o*) recalls in form and position the opercular cell of the antheridium of the Polypodiaceae, but apparently is formed here before the central cell is cut off. In one of the lateral cells a horizontal division is usually (perhaps always) formed, so that the central cell is surrounded by five parietal cells, one basal (*b*), one apical (*o*), and three lateral ones. The central cell now divides by an approximately vertical wall, and these cells divide twice by walls at right angles to each other, so that eight sperm-cells are formed. From the nucleus of each cell, in the usual way, the body of the spermatozoid is formed. In some cases it looked as if only four sperm-cells were formed; but this was not certain. The dehiscence of the antheridium and the free spermatozoids were not seen, but probably the latter resemble those of *Salvinia*. To judge from the appearance in the nearly ripe antheridium, they do not possess

[1] loc. cit. Fig. 2.

more than two complete coils. Their small size renders them unfavourable for a study of the details of development, and no attempt was made to study these.

The ripe prothallium remains completely imbedded in the substance of the massula (Fig. 28), and probably the spermatozoids escape by a softening of the outer surface of the massula which has a corroded appearance in microtome-sections, quite different from the distinct outline of the younger ones.

A comparison of the antheridium with that of other forms does not show a very close resemblance to any. From *Salvinia* it differs in the complete surrounding of the sperm-cells by the parietal cells, and in the separation of the sperm-cells into two groups in the latter. Among the homosporous Ferns, the antheridium of the Hymenophyllaceae, perhaps, resembles it most nearly, especially in regard to the arrangement of the parietal cells. In some cases a triangular opercular cell was observed which, from its position, looked as if it had been formed subsequent to the formation of the vertical walls, much as in *Osmunda*.

Germination of the Macrospores.

The study of the germinating macrospores involves various difficulties. First, to collect a sufficient number it is necessary to collect a large number of plants, as each fertile one furnishes usually only one or two spores, and only a comparatively small number of plants have them at all. The spores only germinate after they have been set free by the decay of the indusium, and the best way to get a supply is to collect a number of fruiting plants and allow them to remain in water until the fertile branches die. These will then finally sink to the bottom of the vessel, and may be picked to pieces and the spores separated. Spores secured in this way will usually germinate promptly, but there is considerable difference in this respect; and, as there is nothing to indicate whether or not germination has begun, it was only by making repeated sowings and sectioning a very

large number of spores that, finally, it was possible to get all of the stages. There is a good deal of difficulty in satisfactorily sectioning the youngest stages, too; but a sufficient number of successful preparations were finally secured to make out clearly the earliest divisions in the prothallium, which Berggren, who alone has studied the female prothallium, failed to get. Berggren's account of the prothallium is extremely imperfect, and is confined entirely to the later stages, and it was largely to determine the early stages that the work was first undertaken.

The ripe macrospore does not become entirely free from the sporangium, the upper part of which remains covered by the cap-shaped upper part of the indusium. The more delicate lower part of the indusium, and the sporangium-wall, rots away and leaves the epispore exposed. From this the filamentous appendages stand out, very much as the glochidia do from the massulae; and when the latter come in contact with the macrospore, the anchor-like ends of the glochidia become entangled in the filaments, and the massulae remain thus firmly attached to the macrospore. Of course this brings the germinating microspores close to the macrospore and facilitates fertilization.

The most prompt germination of the macrospores was found in material gathered in early autumn, which is probably the ordinary time for germination. Spores collected late in November germinated, but less promptly. In all cases there is a good deal of variation, so that it is impossible to state positively just how long is required. In a few cases, within eleven days from the time that the spores were freed from the plants and placed in fresh water, the young plants had already broken through the prothallium, and usually within two weeks this was the case. Probably the first divisions of the prothallium may occur within two or three days, and the whole development be completed within a week, but this is only an approximation, as there is no means of telling the stage of development without killing the prothallium.

A section of the ripe spore, while still within the sporangium, shows its contents to be nearly uniform. The granular protoplasm is arranged somewhat reticulately, and in the living spore there is a good deal of oil, which is dissolved out in the process of imbedding. Besides this there are numerous albuminoid bodies of varying sizes, that stain deeply with alum-cochineal. The nucleus (Fig. 40 *n*) is at the top of the spore-cavity, and is not at all conspicuous. It is somewhat elongated and quite uniform in structure, apparently having very little chromatin, and scarcely staining at all. No nucleolus can be seen.

The first indication of germination is an increase in the size of the nucleus, which becomes nearly globular, and, at the same time, it shows more coarsely granular contents, and a small nucleolus becomes evident (Fig. 41). At this time the cytoplasm in the vicinity of the nucleus becomes free from large granules, and this is the first indication of the position of the mother-cell of the prothallium.

While we have no observations on the first divisions in the prothallium of *Azolla*, there have been some partial observations on that of the related *Salvinia*. Prantl[1] succeeded in demonstrating that the first division was by means of a distinct cell-wall, and that there was no formation of free cells such as Juranyi[2] describes. Prantl, however, failed to get the immediately following stages, although from a study of the older prothallia he was able to tell with considerable accuracy the succession in which the earlier walls were formed.

The earliest stage obtained by me showed the very recent separation of the prothallium mother-cell (Fig. 42). This was a small lenticular cell, whose contents were more uniformly granular than that of the body of the spore. The division-wall was delicate, but easily seen. The nucleus stained deeply, much more so than that of the undivided spore, and had a much larger and more distinct nucleolus. The other nucleus was

[1] Zur Entwicklungsgeschichte des Prothalliums von *Salvinia natans*, Bot. Zeit. 1879, p. 425.

[2] Ueber die Entwickelung der Sporangien u. Sporen der *Salvinia natans*, p. 18.

quite as large and closely resembled it. In this respect *Azolla* offers a strong contrast to *Marsilia*[1], where the prothallial nucleus is much larger than that of the spore. How it compares with *Salvinia* in this respect can only be known after stained sections of the latter have been studied. Berggren[2] failed to demonstrate the presence of a primary division-wall, and figures the earlier stages of the prothallium as having the lower cells of very indefinite form with no distinct wall separating them from the spore-cavity. The first division-wall in the prothallium seems to correspond with that in *Salvinia*. This is a vertical wall (Fig. 44 *I–I*), and divides the cell into two cells of unequal size. In *Salvinia*, according to Prantl[3], the former cell remains sterile, while in *Azolla* it also may produce archegonia, although later than the rest of the prothallium. In a very young prothallium, having but three cells (Fig. 43), the next wall was also nearly vertical, but in other cases (Fig. 46) it looked as if this were not always the case, but that, as in *Salvinia*[4], the second wall was horizontal and divided the larger cell into two nearly equal ones. From the upper one the first archegonium is developed at a very early stage. Its position varies a good deal, depending apparently upon the position of the first division-wall in the prothallium, and also upon the time when the first horizontal wall is formed. If the latter is formed early, the first archegonium is nearly central, but if this is not formed until after two vertical walls have been produced, the archegonium is nearer the side opposite the first cell cut off. In the few cases where successful cross-sections of very young prothallia were made, the archegonium mother-cell was decidedly triangular in outline, indicating that it is cut off by the walls meeting at nearly equal angles (Fig. 52). It is easily distinguished in the very young prothallium by its denser contents that stain more strongly than those of the surrounding cells. The archegonium-mother-cell divides now into two by a transverse wall, the lower of the

[1] Campbell. On the Prothallium and Embryo of *Marsilia vestita*, Proc. Cal. Acad. Sci. 1892, p. 196.

[2] loc. cit. p. 2. [3] Prantl, loc. cit. p. 427. [4] loc. cit. p. 429.

two cells giving rise directly to the egg and the canal-cells, the upper to the neck, no basal cell being formed. In this it agrees with the other heterosporous Pteridophytes.

Up to the time that the first division in the archegonium is completed, the whole prothallium has increased somewhat in size, but this has been entirely at the expense of the spore-cavity, and the exospore has remained intact. The central cell of the archegonium is separated by a single layer of cells only, from the spore-cavity. The young prothallium at this stage (Fig. 47) recalls quite strongly that of *Pilularia* at a similar stage, but also agrees closely with Prantl's account of *Salvinia*. Berggren's figures[1] of prothallia of *A. caroliniana*, at a stage presumably about the same, are too diagrammatic to allow of a satisfactory comparison. They represent the prothallium as composed of perfectly uniform cells arranged in rows converging at the top, where a very small archegonium mother-cell is shown. The whole is totally different from anything observed in *A. filiculoides*.

Shortly after the first division in the archegonium, a rapid increase takes place in the size of all the cells of the prothallium, by which it expands and ruptures the exospore, which breaks open at the top into three lobes corresponding to the three converging lines that mark it at that point.

The most remarkable difference observed between *Azolla* and the other Hydropterideae is the history of the lower of the two nuclei resulting from the division of the primary nucleus of the macrospore. In the Marsiliaceae this remains undivided, and in the later stages seems to become more or less completely disorganized. In *Azolla*, however, where it is quite equal in size to the nucleus of the prothallium mother-cell, it undergoes repeated division, the resulting nuclei remaining imbedded in the protoplasm of the upper part of the spore-cavity, in close proximity to the cells of the under side of the prothallium (Fig. 47 *n*). While the albuminous granules become larger in the lower part of the cavity, the upper nucleated protoplasm loses them almost entirely, and in

[1] loc. cit. Figs. 7 and 9.

stained sections contains only very small colourless granules. The amount of this finely granular protoplasm increases very much in quantity as the prothallium grows. This nucleated protoplasm is evidently concerned in the elaboration of the reserve food-stuff in the spore, in order to facilitate its absorption by the growing prothallium, and later by the embryo. These nuclei have a small nucleolus, and are quite rich in chromatin. The nuclei usually remain free in the protoplasm, but in exceptional cases indications of cell-formation were seen, resembling closely the secondary 'endosperm' in the macrospore of *Selaginella*, and no doubt homologous with it (Fig. 53 *en*). Nothing of a similar kind is known to exist elsewhere; although, in all probability, a careful examination will show the same state of things to obtain in *Salvinia*. That a similar behaviour of the nucleus is not found in the Marsiliaceae, may perhaps be explained by the extremely rapid development and small size of the prothallium in the latter, and the more intimate connexion of the embryo with the cavity of the spore.

The base of the prothallium, which at first is strongly convex, gradually becomes straight as the basal cells expand laterally (Fig. 49), and later, with the vertical growth of the cells, becomes strongly concave, this being especially marked in the older prothallia that have remained unfertilized (Fig. 60).

At the time that the first archegonium is ripe, the prothallium seen in longitudinal section appears nearly hemispherical, but somewhat narrower at the base owing to the lateral growth of the middle cells (Fig. 51). The central cell of the archegonium is separated by but one (or occasionally two) layers of cells from the spore-cavity, and the neck projects considerably above the upper surface of the prothallium. But very little chlorophyll is to be seen at this stage, and even in the older prothallia but very little is found as compared with *Salvinia*, or with the old unfertilized prothallia of the Marsiliaceae.

As the growing prothallium pushes up, it penetrates the

central conical mass of the epispore at the top of the spore, which here seems to become soft; Fig. 50 shows a cross-section of this mass with the top of the archegonium showing through the ragged triangular opening in the epispore. In this way the opening of the archegonium is left free for the entrance of the spermatozoids.

Cross-sections of the prothallium are more or less decidedly triangular, with one angle longer than the others (Fig. 54). This longer angle corresponds to the 'sterile third' of the prothallium of *Salvinia*, and represents the first cell cut off from the prothallium-mother-cell.

In case the first archegonium is fertilized at once, no others seem to be formed; but in the great majority of cases examined by me, the first archegonium was not fertilized, and a varying number of secondary ones had been formed. The first of these arises close to the primary one; indeed, its central cell is generally separated from that of the primary one by but a single layer of cells. The third arises near the base of the larger lobe (Fig. 54 *a* 3). In case all of these remain unfertilized, others arise between them, apparently without any regularity, as any superficial cell apparently can give rise to an archegonium. Nothing resembling the regular meristem of *Salvinia* could be detected, and after the first three, the other archegonia seemed to arise indifferently at any point in the upper surface of the prothallium.

The Archegonium.

The archegonium-mother-cell becomes early distinguished by its larger size, denser contents, and larger nucleus, from its neighbouring cells. It varies a good deal in size and shape, and the later-formed ones are decidedly smaller than the first and second. Sometimes the mother-cell is short and square at the bottom, and sometimes deep and narrow with a pointed lower end. Its development corresponds closely to that of the other Filicineae, especially *Salvinia* to which it bears a very strong resemblance. As in the primary archegonium, no basal

cell is formed, but the first division separates the neck from the central cell (Fig. 56). The neck-canal-cell is formed much earlier than given by Pringsheim for *Salvinia*[1], and is cut off from the central cell about the time that the vertical walls in the neck-cell are formed. The wall by which the neck-canal-cell is separated from the central cell, is concave, but becomes nearly straight as the neck lengthens. Each of the four primary neck-cells divides into four as in *Salvinia*, and like it, the divisions are completed before any marked elongation of the neck is noticeable. The ventral canal-cell is cut off by a very strongly curved wall, and sometimes, if not always, before the divisions in the neck-cells are completed (Fig. 58 *b*). The nucleus of the neck-canal-cell may undergo division, very much as in the homosporous Filicineae and *Isoetes* (Fig. 58). This has not been recorded for *Salvinia*. Whether it always occurs in *Azolla* it is impossible now to say. After the divisions are completed the neck rapidly lengthens and projects strongly above the surface of the prothallium, resembling much more that of the homosporous Filicineae than it does that of the Marsiliaceae.

A curious fact noted was the retention for a long time of an apparently normal structure of the protoplasm and nucleus of the central cell of the unfertilized archegonia. Instead of shriveling up, and the walls of the central cell turning brown as is usually the case, the cell remained turgid, and the protoplasm and nucleus retained much the same structure as in the freshly opened archegonium. Indeed it was often impossible to tell whether an archegonium had been fertilized or not.

The prothallium seems to have very little power of independent existence, and develops but little chlorophyll even in the older unfertilized ones. No root-hairs were observed in any case, and the limit of its growth is probably determined by the amount of food material in the spore. The number of archegonia may occasionally exceed twelve, and from

[1] Goebel: Outlines, p. 232.

eight to ten is not at all uncommon. Sometimes, in very old prothallia little elevations are formed projecting upward between the older archegonia, and upon these small archegonia are developed.

To judge from Berggren's[1] figures of *A. caroliniana*, the prothallium in that species is decidedly larger than in *A. filiculoides*, but the archegonia are much less numerous.

The Embryo.

Berggren made out correctly the first divisions in the embryo, but did not trace in detail any but the very earliest ones, and his figures, as in the case of the prothallium are too schematic to show the cell-arrangement with any degree of accuracy.

The fertilized egg, previous to its first division, elongates vertically. The first, or basal wall, is usually horizontal[2], instead of vertical as in all the other Leptosporangiatae yet investigated. In a very few cases (Fig. 65), the basal wall was nearly vertical, but this was exceptional. From the upper cell the cotyledon and stem arise; from the lower, the foot and first root. Thus the position of the primary organs of the embryo is the same with reference to the basal wall, as in the other Leptosporangiates, but different as regards the archegonium.

The next divisions, as in other cases, divide the embryo into four nearly equal cells. These quadrant-walls (*II*) do not always arise simultaneously. In the only case where a three-celled embryo was found by me, the upper cell was divided. Berggren[3] states that in *A. caroliniana*, it is the lower cell that first divides. Probably it is not always the same one. The formation of the quadrant-walls determines the primary organs. In the upper cell one quadrant gives rise to the stem, and one to the cotyledon; and of the two

[1] loc. cit. Figs. 4–16.

[2] That is, assuming the axis of the archegonium to be vertical.

[3] loc. cit. p. 4.

lower, the one next the leaf-quadrant forms the root, the other the foot; so that these organs have the same relative positions as in the embryo of the ordinary Ferns. Berggren[1] states that the root does not arise until later, and is derived from the foot; but in sections it is plainly recognizable from the very first, and corresponds in position exactly with that of other Ferns.

In regard to the next walls there is not always absolute regularity. In all but the stem-quadrant, the octant-walls divide the quadrants into exactly equal parts, and this may be true also of the stem-quadrant. In the latter, however, (Fig. 64 *a*), the octant-wall may make an acute angle with the quadrant-wall, and the larger cell of the two thus formed functions at once as the apical cell of the stem, and divides from now on by walls directed alternately right and left. When the stem-quadrant is divided into equal cells by the octant-wall it is probable, although this was not positively proved, that for a time the apical cell of the stem forms three sets of segments instead of two. This seems probable from the fact that often when seen from the side (see Fig. 71 *a*), two series of segments can be seen, which could hardly be true were there but two series cut off from the apical cell.

The Cotyledon.

The first divisions of the cotyledon are extremely regular, and resemble those in the later leaves. The cotyledon, however, as well as the other leaves of the young plant, is not divided into the lobes found in the leaves of the older plants. Following the median octant-wall, a vertical wall is formed in each of the two octant-cells (Fig. 66 *b*), forming two cells that seen in section appear triangular, and two which appear to be four-sided. The two former, which have larger nuclei than the other cells, divide for some time in much the same way that we saw in the formation of the sporocarps in the fertile lobes of the sporophylls, and may, perhaps, be equally

[1] loc. cit. p. 4.

well designated apical cells. The other cell in each octant divides by tangential and radial walls arranged with a good deal of regularity. By the growth of the two initial cells (x, x′) the young cotyledon rapidly grows at the lateral margins, and it bends forward so as to partly include the stem-apex. At the same time the upper marginal cells divide rapidly by oblique walls alternately on the inner and outer side, so that the cotyledon also grows in height. By this time the cotyledon has become about four cells thick.

The Stem-Quadrant.

As we have seen, the divisions in the stem-quadrant are not perfectly uniform. In case a two-sided apical cell is established at once, it divides from this time very much as in the mature plant. Each segment divides into a ventral and dorsal half, and each of these again into an acroscopic and basiscopic cell. In case the first division in the stem-quadrant divides it equally, it is not possible to say which of the cells will become the apical cell of the stem, but this is determined by the first division in each cell. One of the cells divides by a vertical wall into equal parts, and becomes the second leaf; the other, as already indicated, forms regular segments. When the octant-cells are unequal, the smaller of the two, which may be considered as the first segment of the apical cell of the stem, becomes the mother-cell of the second leaf. At the base of the first leaf, between it and the stem, a group of short hairs (Fig. 71 *h*) is formed at an early stage.

The Root.

The primary root in *Azolla* arises in exactly the same way as in the other Leptosporangiatae. After the first division of the root-quadrant, one of the resulting octants becomes at once the apical cell. The first segment is usually cut off parallel to the basal wall of the embryo, and the next strikes it and the octant-wall (Fig. 66 *c*) so that the apical cell lies at this stage close to the octant wall. In the other octant of the

root-quadrant, the divisions are irregular, and its limits are soon merged in those of the foot. From the first the divisions in the root take place with great regularity. After one complete set of lateral segments has been cut off, a cell is cut off from the outer face of the apical cell, forming the root-cap. Unlike other Ferns, this is the only cap-cell cut off, and all the other segments are lateral. The cap-cell divides later into two (Fig. 71 *b*), and in these cells divisions continue to form where they join the rest of the epidermis, so that the young root is enclosed in a sheath formed of two layers of cells (Fig. 73). The lateral segments of the apical cell are shallow and arranged very symmetrically. The first wall in each divides it into an inner and outer cell, and from the latter is later developed the fibro-vascular bundle.

The Foot.

The divisions in the foot are more regular than is usually the case. This is especially noticed when sections are made parallel to the quadrant-wall (see Figs. 66, 72). The general arrangement of the cells is much like that of the cotyledon, but the divisions are much less numerous, especially the transverse walls, and the cells are therefore larger and more elongated. Corresponding with the upward growth of the cotyledon, there is an elongation of the foot, so that the base of the foot extends downward much beyond the base of the root-quadrant, which thus comes in the older embryo to appear lateral, and no doubt led to Berggren's mistake of supposing that the root originated from the foot.

The Subsequent Growth of the Embryo.

The second leaf arises practically at the first segment of the apical cell of the stem, and each succeeding segment gives rise to a leaf. The longitudinal growth of the root is slow, although a large number of segments may be cut off from the apical cell; but these remain flattened, and only elongate at a late stage in the development of the embryo.

The fibro-vascular bundles are poorly developed and arise

comparatively late. No trace of them can be seen until the second leaf is pretty well advanced. There is nothing peculiar about their development. Simultaneously in the axis of the root and stem, and extending into the centre of the cotyledon, a series of longitudinal walls arise that give rise to a thin procambium-cylinder. In the axis of this the cells become transformed into tracheids with close spiral thickenings in their walls. At the point of junction of the primary bundles the tracheids are as usual irregular and connect the tracheary tissue of the three bundles. No trace of a bundle could be detected in the foot. The development of the fibro-vascular bundles does not take place until some time after the embryo has broken through the prothallium.

The second root arises close to the base of the second leaf, and originates from single epidermal cell in the same way that the later ones do (Fig. 70 *r'*). A rapid growth takes place now in all the cells of the embryo, and in the cotyledon intercellular spaces are formed, which filling with gases, soon cause the young plant to rise to the surface of the water. As the embryo breaks through the episporic appendages at the top of the spore, these are forced apart, and the top of the indusium, which has covered it up to this time, is thrown off. The young plant at this stage becomes very easily separated from the prothallium, and is often found floating free. At this stage the cotyledon forms a sort of bell-shaped sheath, opening only in one side by a narrow cleft, and completely surrounding the growing-point within. The root is still inconspicuous, and forms merely a slight protuberance on one side of the foot which has the form of a short cylindrical stalk. The stem is at right angles to the foot, and the succeeding leaves form, as in the mature plant, two ranks, overlapping and completely hiding the stem.

The growth of the first root is limited, and it is distinguished from the later ones by peculiar short root-hairs which stand out stiffly from it (Fig. 75). The succeeding roots, except the second, are formed considerably later, and there does not seem to be any determined point at which they arise.

The mesophyll of the cotyledons and the leaves immediately following does not show the peculiar elongated cells found in the later leaves, and these first appear in about the fourth leaf, but are not as well developed as in the later ones. In all of the leaves, however, with the exception of the cotyledon, the peculiar cavity, filled with a colony of *Anabaena*, is to be seen.

The *Anabaena* begins to grow almost as soon as the first divisions in the embryo are completed. If a young embryo is dissected out, it will be found that in the space between the cotyledon and the stem, that a number of very short *Anabaena*-filaments are present. As the embryo pushes up through the space between the archegonium and the indusium, the *Anabaena*-cells collected there are carried up with it, and then begin to grow. They assume the blue-green colour of the active cells, elongate and divide rapidly by a series of transverse walls into short filaments that at first look like an *Oscillaria*. Very soon, however, the cells round off, heterocysts are formed, and the typical form of the ordinary filaments is attained. Some of these remain tangled about the growing point of the stem, while others creep into the cavities at the base of the leaves.

No branches are formed in the young plant before about eight or ten leaves are produced. Whether the position of the first branch is constant, I cannot say, as the point was not critically examined.

Conclusion.

A comparison of the development of *Azolla* with other forms does not show a very close resemblance to any one, and indicates a somewhat isolated position for the genus. Its nearest ally is unquestionably *Salvinia*, with which it agrees in the general plan of its growth, the two-sided apical cell of the stem, and especially the development of the sporocarp. However, as Strasburger has shown, except the first divisions of the apical cell, the resemblance is not very close, and in the decidedly apical growth of the leaves and the absence of

bipartition, *Salvinia* offers a strong contrast to *Azolla*, as of course it does in the absence of roots. This latter is, however, probably of secondary importance, as the roots are replaced by the peculiar submerged leaves.

The origin and development of the sporocarp, as well as the structure of the spores, is too similar to be accounted for except by the assumption of a relationship. The massulae and the episporic appendages of the macrospore differ only in degree from those of *Salvinia*, where all the microspores are held together in one mass, and where the epispore of the macrospore is less developed. As indicated in the account of the macrosporangium of *Azolla*, there is every reason to believe that it is derived from some form with more than one macrosporangium in the sorus. In regard to the embryo, that of *Azolla* in its younger stages corresponds very closely to those of *Salvinia*[1]. Indeed, so close is the correspondence that one is inclined to regard the embryo of the former as not really rootless, as the very first divisions in what corresponds to the root-quadrant of *Azolla* are very like. A further investigation of this point, by careful microtome-sections, is very much to be desired.

The prothallium and sexual organs of *Azolla* are also extremely like those of *Salvinia*, the most noticeable difference being the much greater size and regular meristem in the female prothallium of the latter. Whether the endosperm-nuclei are present in *Salvinia* remains to be seen.

Compared with the Marsiliaceae there is much less resemblance. The sporocarps are very different, being in the Salviniaceae single sori, while in the Marsiliaceae, each fruit is a whole leaf-segment with several sori. The prothallium is much less reduced, especially the female one, and the archegonia more nearly resemble those of the homosporous Ferns, both in the length of the neck and the division of the nucleus of the neck-canal-cell. A very noticeable difference, and one which must be regarded probably as an important one, is the presence of the endosperm-nuclei.

[1] Sadebeck in Schenk's 'Handbuch,' vol. i. p. 217.

When *Azolla* is compared with the other Pteridophytes, it is evident enough that its nearest affinities are with the homosporous Filices. We have already indicated the very clear resemblance to these in the development of the sporangia and the indusial nature of the sporocarp wall. When a nearer comparison is made it seems probable that it is with the lower members of the Leptosporangiate series that its affinities are most marked. The form of the indusium recalls some of the Cyatheaceae and Hymenophyllaceae, and the leaves in their earlier stages resemble those of some of the simpler forms in the latter family.

We may conclude, then, that the two families of the Hydropterideae represent the ends of two different lines of development. Of these the Salviniaceae have been derived from the lower members of the Leptosporangiate series, possibly from near the Hymenophyllaceae, and that the Marsiliaceae have arisen from forms more like the Polypodiaceae. Of the two families, the Salviniaceae have departed less from the parent stock in regard to the reduction of the sexual generation, but the sporophyte is much less like that of the ordinary homosporous forms than that of the Marsiliaceae.

The two genera of the Salviniaceae differ much more from each other than do those of the Marsiliaceae, and it is not at all likely that one form has been derived from the other, but that the two genera diverged at an early stage in the development of the line.

EXPLANATION OF FIGURES IN PLATES VII, VIII, AND IX.

Illustrating Professor Campbell's paper on *Azolla filiculoides*, Lam.

The Figures are all drawn from microtome-sections, except Figs. 27 and 75.

PLATE VII.

Fig. 1. A horizontal section through the growing-point of a branch of *Azolla filiculoides*. ×350. Owing to the upward curvature of the apex, the apical cell was not cut through in this section. *L*, leaves; *K'n*, young branch; *h*, hair; *sp*, young macrosporangium.

Fig. 2. A horizontal section through the apex of a similar branch. ×600.

Figs. 3 and 4. Vertical longitudinal sections through the apex of the stem. ×600. ×, the apical cell; *n*, *Anabaena*-filaments; *b*, a hair; *r*, mother-cell of a root.

Fig. 5. Three successive transverse sections just behind the apex. ×600. *m*, the median wall; *L*, mother-cell of a leaf.

Fig. 6. An older transverse section. The leaf already shows the two lobes, *L* and *L'*.

Fig. 7. Single lobe of a young sterile leaf. ×600.

Fig. 8. Fertile segment of a leaf, showing two very young sporocarp-rudiments. ×600. The apical cells have the nuclei indicated.

Fig. 9. Young female sporocarp. The archesporium is already differentiated. *id*, indusium. ×600.

Figs. 10 and 11. Older stages of the same. ×600. *t*, the first tapetal cell.

Fig. 12. Transverse section of a stage somewhat older than in Fig. 11. ×600. *t*, tapetum; *w*, wall.

Figs. 13–16. Older stages of the sporocarp. ×350. The nuclei of the tapetal cells are indicated, the sporogenous cells are shaded. *n*, *Anabaena*-filaments that have crept into the open indusium. *sp* (Fig. 16), rudimentary sporangium. In Fig. 16, the isolation of the spore-mother-cells has begun.

Fig. 17. A single spore-mother-cell, just before its separation from the others. ×1500.

Fig. 18. Two spore-mother-cells, showing the beginning of the first division. The nuclear spindle is well marked; *a*, from the pole; *b*, from the side. ×750.

Fig. 19. Two later stages. In *a*, the first division is nearly completed. In *b*, the second division is complete, and the division into the four spores has begun. ×750.

Fig. 20. The four spores lying within the mother-cell. ×750.

Fig. 21. Young macrospore surrounded by the nucleated protoplasm of the disintegrated tapetum. ×750.

Fig. 22. Longitudinal section of young microsporangial sorus. × 100. *col.* columella.

Fig. 23. Young microsporangium. × 350.

Fig. 24. Cross-section of a nearly ripe microsporangium, cutting through two massulae. The forming glochidia lie in the space between: *n*, tapetal nuclei. × 250.

Fig. 25. Ripe microspore; *a*, in section; *b*, from above.

PLATE VIII.

Fig. 26. Nearly ripe microsporangium. × 250.

Fig. 27. A single free massula. × 250. *sp*, spores; *gl*, glochidia.

Fig. 28. Section through a massula containing the prothallia. × 350.

Figs. 29–33. Development of the male prothallium. × 560. *I*, first wall; *b*, basal cell of antheridium; *o*, opercular-cell.

Fig. 34. Transverse section of a ripe antheridium. × 750. *a*, a top view; *b*, median section.

Fig. 35. Median longitudinal section of a ripe macrosporangial sorus. × 100. *sp*, sporangium-wall; *n*, *Anabaena*-cells; *s*, episporic appendages; *ex*, part of the exospore that has become detached; *ep*, lower epispore: *id*, indusium.

Fig. 36. Median section through the episporic bodies at the top of the spore. × 350. ×, one of the three pear-shaped bodies of the so-called 'Schwimm-apparat'; *n*, tapetal nuclei. The remains of the sporangium-wall is seen above, with *Anabaena*-cells lying over it.

Fig. 37. Section, parallel to the surface of the spore, of the lower epispore. × 350.

Fig. 38. Median longitudinal section through the base of the sporocarp. × 350. *ep*, epispore; *ex*, exospore; *sp*, sporangium-wall.

Fig. 39. Transverse section of the episporic appendages at the top of the spore. × 100.

Fig. 40. Longitudinal section through the upper part of the ungerminated macrospore. × 600. *ex*, exospore; *n*, nucleus.

Fig. 41. Transverse section through the macrospore at the beginning of germination. × 600. *nu*, nucleolus.

Fig. 42. First division in the germinating macrospore. × 350. *pr*, prothallium-mother-cell.

Figs. 43–49. Development of the female prothallium, seen in vertical action. × 350. Figs. 44 and 46 are diagrams of 43 and 45. *b*, the basal wall. The others are numbered. *ar*, archegonium. In Fig. 48 *a*, *b*, and *c* are consecutive sections of the same prothallium.

PLATE IX.

Fig. 50. Young prothallium breaking through the epispore. × 250.

Fig. 51. Full-grown prothallium in vertical section. × 350. The archegonium has not been fertilized.

Figs. 52, 53. Two transverse sections of a young prothallium. × 350. *o*, archegonium; *en*, endosperm.

Fig. 54. Two transverse sections of a prothallium showing three archegonia. × 250.

Fig. 55. Transverse section of an old prothallium with nine archegonia.

Figs. 56–59. Development of the archegonium. × 350. *b*, ventral canal-cell; *c*, neck-canal-cell; *o*, egg.

Fig. 60. Prothallium showing its relation to the spore. *in*, indusium; *sp*, remains of sporangium; ×, upper part of epispore forced aside by the growth of the prothallium. × 100.

Fig. 61. Section of prothallium containing a two-celled embryo. × 350.

Fig. 62. Vertical section of a three-celled embryo. × 350. In this and the succeeding Figures, *b*, indicates the basal wall; *II*, the quadrant-walls; *III*, the octant-walls.

Fig. 63. An eight-celled embryo. × 350.

Fig. 64. Two horizontal sections (*a* and *b*) of a young embryo. × 350. The stem *st* has a two-sided apical cell. *L*, cotyledon; *st*, stem; *f*, foot; *r*, root.

Fig. 65. Vertical section of young embryo, with nearly vertical basal wall *b*—*b*. × 350.

Fig. 66. Three sections of a young embryo, cut parallel to the quadrant-wall. × 350. × – ×′, initial cells of cotyledon.

Fig. 67. Two sections of an embryo of about the same age as in Fig. 66, but cut longitudinally.

Fig. 68. Slightly older stage; the root has formed the cap-cell. × 350.

Fig. 69. Horizontal section of an older embryo passing through the stem-apex. × 350. L^1, L^2, L^3, the three first leaves; ×, apical cell of the stem.

Fig. 70. A similar section of an older embryo, showing the origin of the second root. × 350. *h*, hairs.

Fig. 71. Two longitudinal sections of an embryo, somewhat younger than the one shown in Fig. 64: ×, apical cell of stem; *h*, hair; *L′*, cotyledon. × 350.

Fig. 72. *a*, *b*, *c*, *d*. Transverse sections of an embryo of about the same age. × 350.

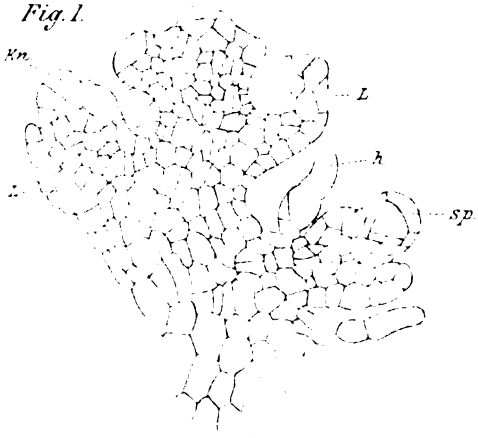
Fig. 73. Longitudinal section of the first root of an older embryo. × 350.

Fig. 74. Longitudinal section of young plant showing the arrangement of the primary fibro-vascular bundles. × 100.

Fig. 75. Young plant still attached to the macrospore (*sp*). × 40. *r*, first root.

v, *d*, ventral and dorsal cells of a segment cut off from the apical cell of the stem.

Fig. 1.



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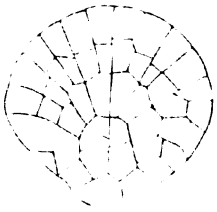

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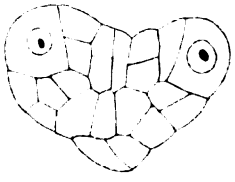

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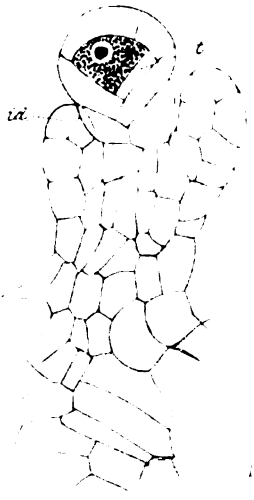

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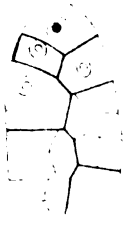

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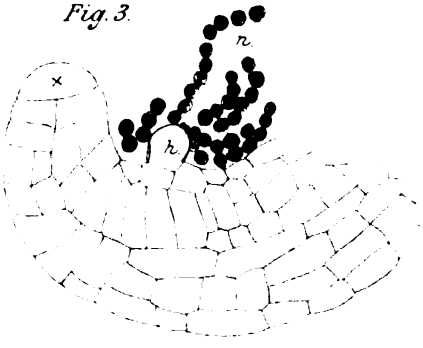

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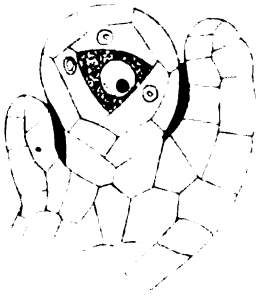

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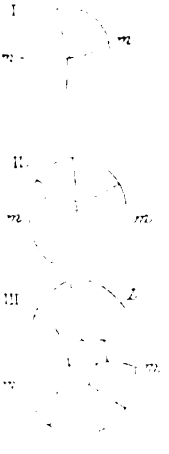

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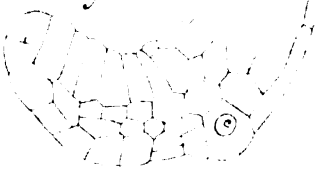

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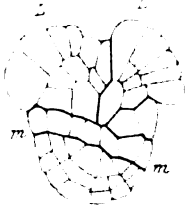

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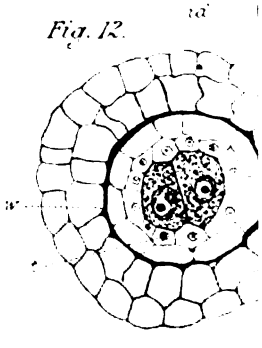

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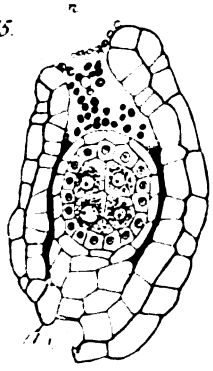

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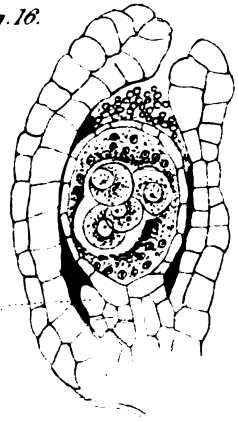

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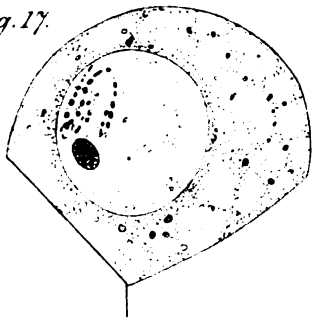
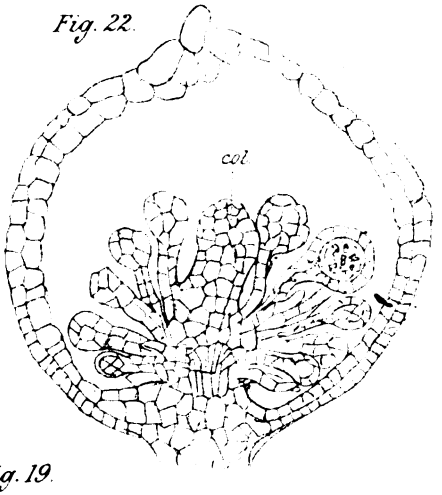


Fig. 13.

Fig. 18.

Fig. 23.

Fig. 22.



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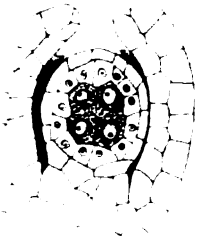

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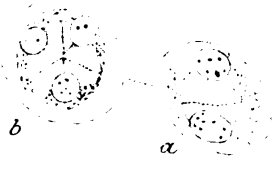

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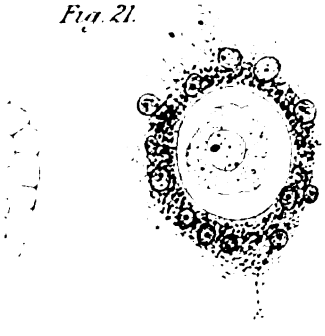

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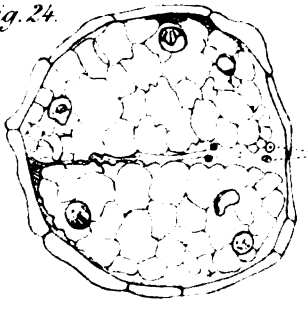

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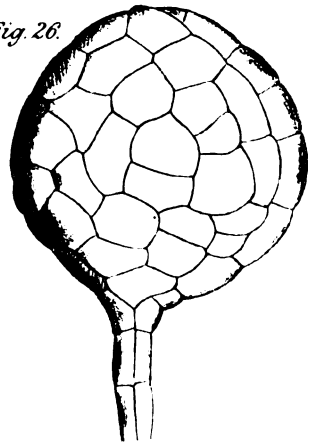

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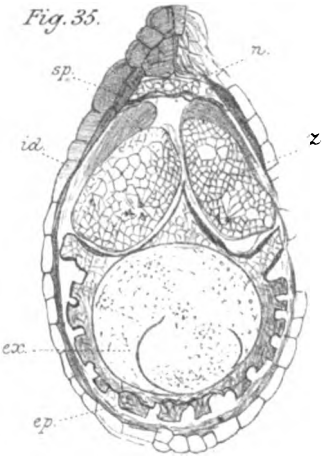

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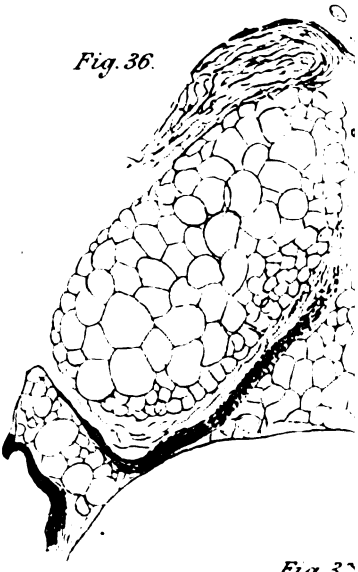

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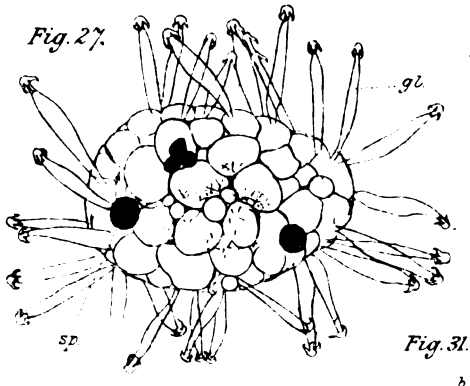

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Fig. 37.

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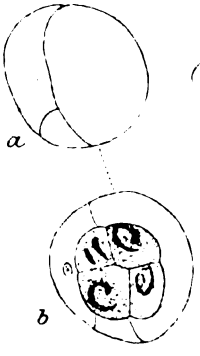

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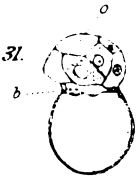

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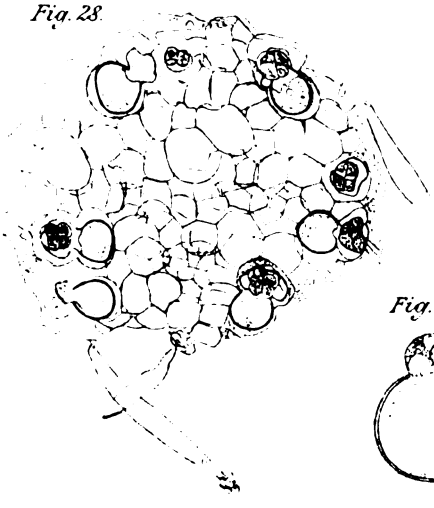

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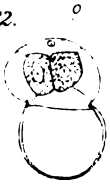

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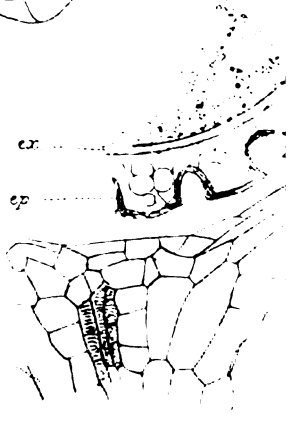

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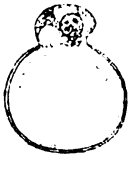

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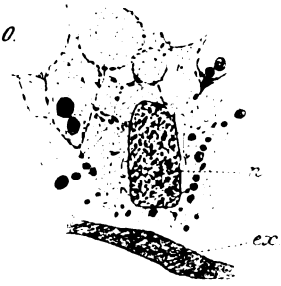

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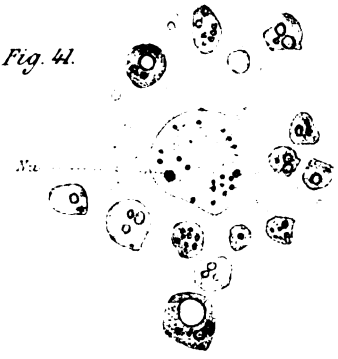

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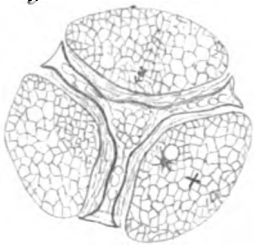

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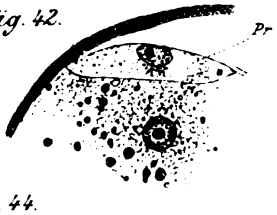

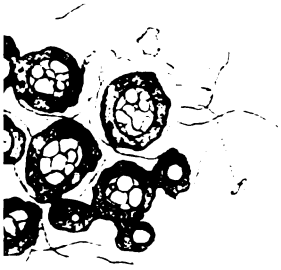

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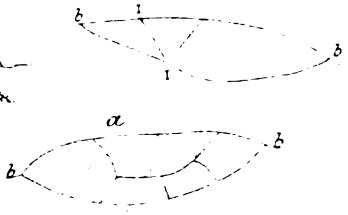

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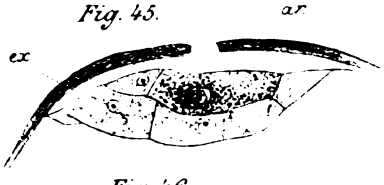

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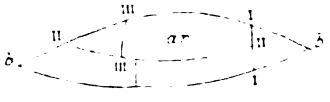

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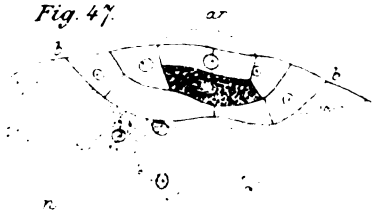

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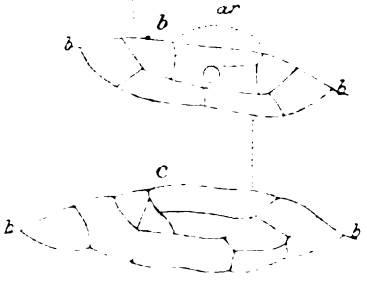

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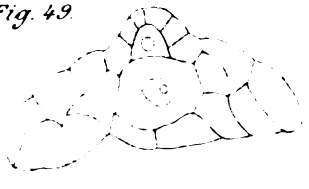

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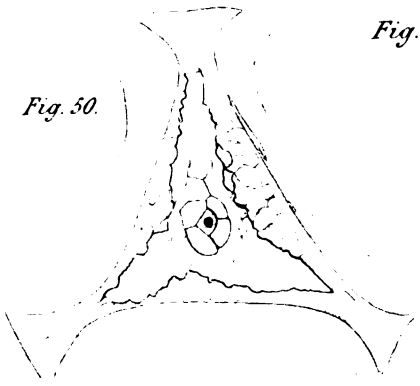

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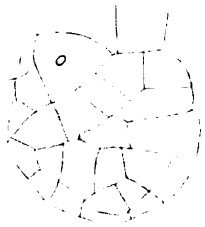

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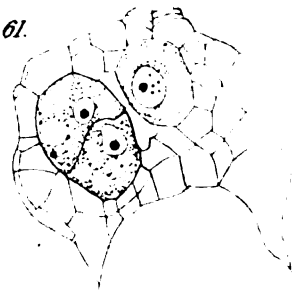

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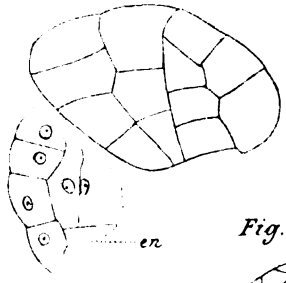

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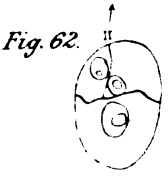

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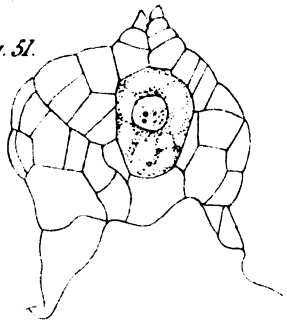

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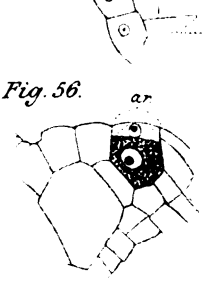

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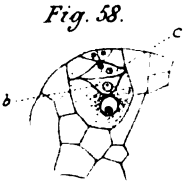
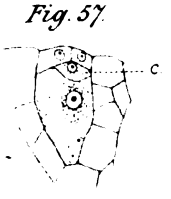


Fig. 58.

Fig. 59.



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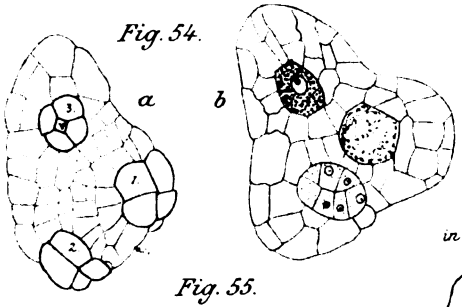
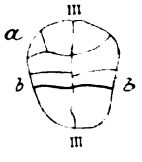


Fig. 54.



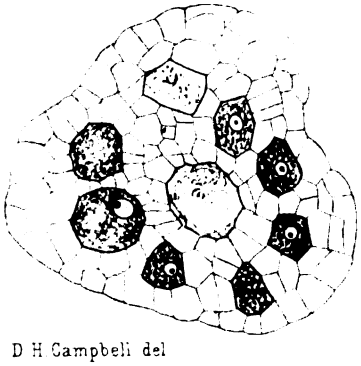


Fig. 55.

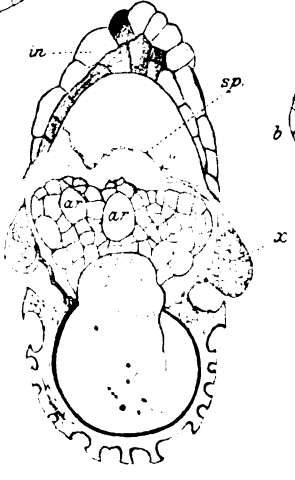


Fig. 60.

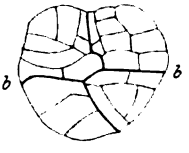


Fig. 68.

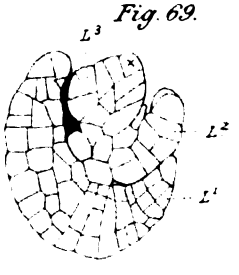


Fig. 69.

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Fig. 64.

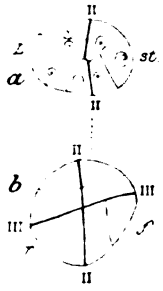


Fig. 71.

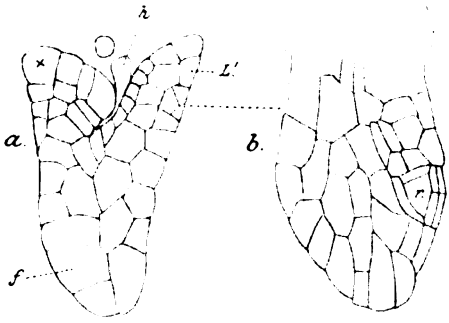


Fig. 73.

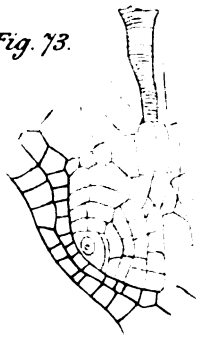


Fig. 63.

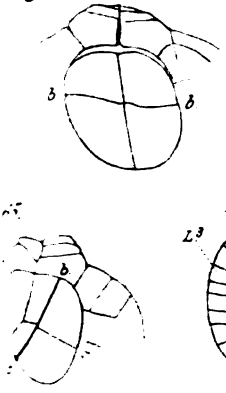
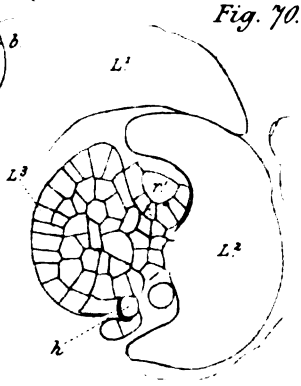
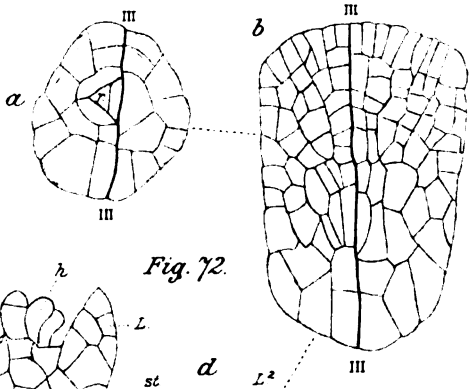


Fig. 70.





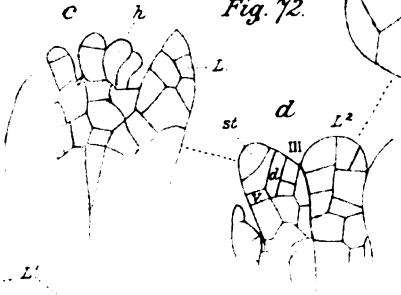


Fig. 72.

Fig. 66.

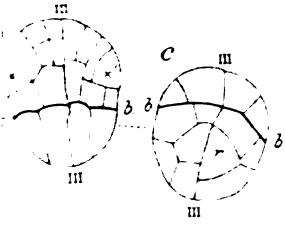


Fig. 74.

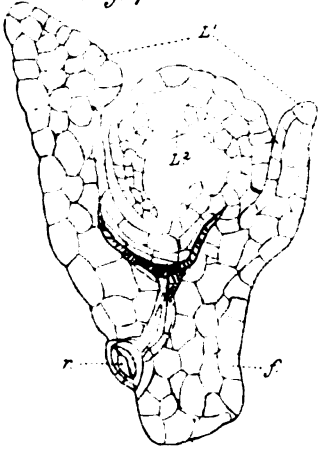


Fig. 67.

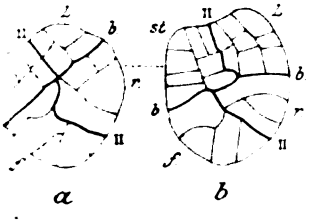
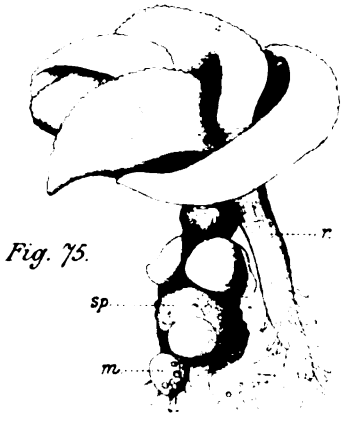


Fig. 75.



University Press, Oxford

A Synopsis of the Genera and Species of Museae.

BY

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Keeper of the Herbarium, Royal Gardens, Kew.

KEY TO THE GENERA.

* *Flowers hermaphrodite.*

1. **Heliconia.** Ovules solitary in the cells. Leaves not distichous. *Tropical America.*
2. **Strelitzia.** Ovules many in each cell. Leaves distichous. Petals very unequal, two connate in a sagittate blade with a narrow haft. *Cape Colony.*
3. **Ravenala.** Ovules many in each cell. Leaves distichous. Petals nearly equal. *Madagascar, Guiana, and North Brazil.*

** *Flowers unisexual.*

4. **Musa.** Flowers of the upper clusters male, deciduous. *Warmer regions of the Old World.*

Genus 1. HELICONIA, *Linn.*

Flowers hermaphrodite. *Sepals* 3, lanceolate, equal; one free, the two others more or less adnate at the base to the corolla. *Petals* united in a unilateral tube which is 3-toothed at the top, and placed opposite the free sepal. Perfect *Stamens* 5, attached high up in the corolla-tube; filaments short; anthers linear, basifixed; sixth stamen represented by a small petaloid staminode

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placed opposite the free sepal. *Ovary* inferior, globose, 3-celled; ovules solitary in the cells, erect; style filiform; stigma capitate. *Fruit* indehiscent, often by abortion 2- or 1-celled. *Seeds* with an intruded testa, firm albumen, and straight embryo.—*Stem* erect, sheathed by the petioles of the non-distichous leaves. *Panicle* formed of several umbels of flowers, placed in the axils of the brightly-coloured lanceolate or ovate branch-bracts. *Flowers* various in colour. *Fruit* small, usually blue. Kuntze employs for the genus the name *Bihai*, used by Philip Miller in 1739 and Adanson in 1771.

Key to the Species.

Subgenus **Platychlamys**. Branch-bracts ovate-acuminate, deeply boat-shaped, as in *H. Bihai*.
- Branch-bracts crowded on the rachis.
  - Branch-bracts ascending . . . . Sp. 1.
  - Branch-bracts spreading . . . . Sp. 2–5.
- Branch-bracts spaced out on the rachis.
  - Branch-bracts very hairy . . . . Sp. 6–7.
  - Branch-bracts glabrous . . . . . Sp. 8–12.

Subgenus **Stenochlamys**. Branch-bracts lanceolate-acuminate, shallowly boat-shaped, as in *H. psittacorum*.
- Leaves large . . . . . Sp. 13–20.
- Leaves small.
  - Leaves green beneath . . . . . Sp. 21–26.
  - Leaves purple beneath . . . . . Sp. 27.
  - Leaves farinose beneath . . . . . Sp. 28–29.

Subgenus **Platychlamys**.

1. **H. episcopalis**, Vell. Fl. Flum. III, t. 22; Peters. in Fl. Bras. III, pt. 3, t. 2; *H. Ferdinando-Coburgi*, Szys. in Wawra, Iter Princ. Sax.-Cob. II, 88, t. 5; *H. biflora*, Eich.; *H. thyrsoidea*, Mart. Whole plant 6–7 ft. long. Leaves oblong, the lower 2–3 ft. long, 8–10 in. broad, rounded at the base, green and glabrous beneath. Peduncle long, stiffly erect, glabrous. Panicle dense, oblong, 3–4 in. long; branch-bracts 6–18, ovate, acute, bright red, glabrous, ascending, much imbricated, 2–3 in. long, 1½ in. round at base, deeply boat-shaped; rachis quite hidden. Flowers 2–6 in a cluster, whitish, under 2 in. long; pedicels

very short; flower-bracts hairy outside. Staminode minute. Brazil, *Blanchet*, 2971! *Glaziou*, 8496! New Granada, *Holton*, 211! *Triana*, 2647! Peru, *Haenke*.

2. **H. imbricata**, Baker; *Bihai imbricata*, Kuntze, Revis. Gen. 684. Whole plant 3–4 yards long, glabrous in all its parts. Leaves oblong, acute, 3–4 ft. long, a foot or more broad. Peduncle sub-erect, nearly a foot long. Panicle dense, conic, 8 in. long, half a foot broad at the base, 2 in. at the top; branch-bracts about 10 on a side, ovate, acute or acuminate, coriaceous, the lowest spreading horizontally, 4 in. long by 4 in. round at the base. Flowers many in a cluster. Costa Rica; Port Lemon, *Kuntze*, who proposes for the species with dense inflorescence a section named *Taeniostrobus*.

3. **H. Mariae**, Hook. fil. in Journ. Linn. Soc. VII, 68; *H. elegans*, Peters. Whole plant 6–7 yds. or more long. Leaves oblong, long-petioled, 3–4 ft. long by a quarter as broad, rounded at the base, green and glabrous beneath. Panicle pendulous; peduncle stout, glabrous, above a foot long; branch-bracts 20–30 or more, slightly imbricated, ovate, glabrous, very coriaceous, deeply boat-shaped, all more or less reflexed, 2–3 in. long, 2 in. round above the base. Flowers red, 15–20 in a cluster, 1½ in. long; bracts glabrous; pedicels hairy. Staminode lanceolate. Fruit blue. New Granada, province of Bolivar, *Dr. Anthoine*!; Panama, *Wagner*, *Kuntze*. It was named, at the request of the discoverer, in compliment to the Empress of Russia.

4. **H. conferta**, Peters. in Fl. Bras. III, pt. 3, 13, tab. 3, fig. 2. Leaf oblong, rounded at the base, green and glabrous beneath, about 4 ft. long by a foot broad. Peduncle stout, pubescent; panicle nearly a foot long and broad; branch-bracts about 10, cordate-ovate acuminate, 4–5 in. long, 3–4 in. broad at the base, only an inch halfway up, crowded so that they hide the rachis, spreading, glabrous. Flowers 2 in. long, glabrous; flower-bracts oblique ovate. Guadeloupe, *Duchassaing*.

5. **H. Wagneriana**, Peters. in Fl. Bras. III, pt. 3, 13. Leaf oblong, green and glabrous beneath, 4 ft. long, nearly a foot broad, gradually narrowed to the base. Panicle 1½ ft. long by nearly a foot broad; rachis slightly flexuose, pubescent; branch-bracts close, ovate-acuminate, deeply boat-shaped, the lower 5–6 in. long, 3–4 in. round near the adnate base. Flowers

many in a cluster; bracts ovate-oblong, glabrous. Panama, *Wagner*.

6. **H. villosa**, Klotzsch, in Linn. XX, 463; Peters. in Fl. Bras. III, pt. 3, t. 4. Whole plant 6–8 ft. long. Leaves oblong, green and glabrous beneath, 2–3 ft. long by nearly a foot broad, rounded at the base; petiole long, stout. Panicle pendulous from a curved stout peduncle which is densely clothed with soft brown hairs, deltoid, a foot long and broad; rachis flexuose, densely pubescent; branch-bracts ovate, deeply boat-shaped, densely hairy outside, spreading, the central ones 3–4 in. long, 2 in. round above the shortly adnate base, the lowest bract 5–6 in. long. Flowers many in a cluster, 2 in. long; bracts lanceolate, hairy, as long as the flowers. Staminode subulate, minute. Venezuela, *Moritz*, 250! *Fendler*, 1771! Brazil, *Sello*.

7. **H. vellerigera**, Poepp. Reise Chili, II, 295. Leaf unknown. Whole plant 6–7 ft. long. Panicle lax; rachis slightly flexuose, densely clothed with long soft brown hairs; branch-bracts ovate, deeply boat-shaped, densely pubescent, 4–6 in. long, above 3 in. broad above the base, narrowed suddenly above the middle. Upper Peru; province of Maynas, *Poeppig*.

8. **H. Bihai**, Linn. Syst. Veg. ed. XIII, 204; Andr. Bot. Rep. t. 640; Bot. Reg. t. 374; L. C. Rich. Comm. t. 8 and 10, fig. 1; Peters. in Fl. Bras. III, pt. 3, t. 5; *H. caribœa*, Lam.; *H. luteo-fusca*, *nigrescens*, and *variegata*, Jacq. Hort. Schoen. I, 25. Whole plant 8–20 ft. long. Leaves oblong, long-petioled, green and glabrous beneath, the lower 3–4 ft. long, nearly a foot broad. Panicle lax, erect or drooping, 1–2 ft. long; rachis glabrous, hardly at all flexuose; branch-bracts all arcuate-ascending, ovate acuminate, deeply boat-shaped, bright crimson, with a yellow edge, the lowest 5–6 in. long, 2½–3 in. round above the broadly adnate base. Flowers many in a cluster, whitish, 1½–2 in. long; bracts oblong-lanceolate, glabrous; pedicels short. Staminode oblong, acute. Throughout eastern tropical America from the West Indies to South Brazil. Venezuela, *Moritz*, 200! *Fendler*, 1490! Guatemala, *Donnell-Smith*, 1830! Panama, *Hayes*! Santa Marta, *Schlim*, 1000! New Granada, *Holton*! Introduced into cultivation in Europe from the West Indies in 1786. I cannot clearly

separate the Mexican *H. Bourgoeana*, Petersen (Bourgeau, 2502 ! 2609 !), nor the Peruvian *H. Poeppigiana*, Eichler, both of which are fully described in Flora Brasiliensis. *H. indica*, Lam., *H. buccinata*, Roxb., *H. austro-caledonica*, Vieill., and *Heliconiopsis amboinensis*, Miquel (Rumph. Amboin. V, t. 62, fig. 2), appear to be only cultivated forms of this species, of which I have seen specimens from New Caledonia, New Guinea, and the Solomon Islands. *H. aureo-striata*, Bull Cat. 1881, 18, with woodcut, said to be from the South Sea Islands, is, I presume, a form of this with variegated leaves. *H.? triumphans*, Hort. Linden., Ill. Hort. n. s. tab. 448, from Sumatra; and *H.? striata*, Hort. Veitch., Flore des Serres, tab. 2416–7, said to have been received from New South Wales, are garden plants with variegated leaves, not known in flower. *H.? leucogramma*, Hort. Van Houtte, proved to be a *Calathea*. *H. Seemanni*, Hort. Van Houtte, of which the leaves are figured in their catalogue for 1875–6, p. 183, may be also a form of *Bihai* with leaves variegated with white.

9. **H. humilis**, Jacq. Hort. Schoen. I, t. 48–49; Red. Lil. t. 382–3; Hook. fil. in Bot. Mag. t. 5613. Whole plant 4–5 ft. long. Leaves oblong, acute, long-petioled, green and glabrous beneath, 1½–2 ft. long, 4–6 in. broad at the middle, deltoid at the base. Panicle erect or drooping; branch-bracts few, spaced out on the pubescent flexuose rachis, ovate, deeply boat-shaped, glabrous, bright red with a narrow green edge, all more or less ascending, the lowest 4–6 in. long, 2–2½ in. round above the broadly adnate base. Flowers many in a cluster, greenish-white, 2 in. long; bracts glabrous, as long as the flowers. Staminode oblong, acute. Trinidad, *Purdie*, 41 ! *Fendler*, 806 ! 807 ! Upper Amazon, *Traill*, 815 ! Scarcely more than a variety of *H. Bihai*.

10. **H. pendula**, Wawra, Iter Max. 142, t. 21. Whole plant 8–9 ft. long. Leaves oblong, green and glabrous beneath, reaching a length of 4–5 feet and a breadth of a foot long. Panicle pendulous from a long curved peduncle, a foot or more long, half a foot broad at the base; branch-bracts spaced out on the very flexuose pubescent rachis, all except the uppermost reflexed, ovate, deeply boat-shaped, glabrous, bright red with a greenish-yellow margin, 3–4 in. long by under 2 in. round

above the shortly adnate base. Flowers 10–12 in a cluster, yellowish-white, 2 in. long; pedicels short; bracts lanceolate, hairy, as long as the flowers. Staminode lanceolate. Bahia, *Blanchet*, 2984! Santarem, *Spruce*, 445! Bogota, *Turner*!

11. **H. curtispatha**, Peters. in Fl. Bras. III, pt. 3, 15. Leaves not seen. Panicle pendulous; rachis flexuose, pubescent; branch-bracts ovate, deeply boat-shaped, spaced out on the rachis, glabrous, coriaceous, 2–3 in. long, 2 in. round at the base, the lower only reflexed. Flowers many in a cluster, under 2 in. long; pedicels short; bracts ovate-lanceolate, as long as the flowers, hairy on the back. Panama, *Wagner*; Nicaragua, *Seemann*, 169!

12. **H. rostrata**, Ruiz et Pav. Fl. Peruv. t. 305. Whole plant 6–8 ft. long. Leaves oblong, acute, green and glabrous beneath, subcordate at the base, 2 ft. long. Panicle pendent, a foot long, 6–8 in. broad at the base; rachis very flexuose, finely pubescent; branch-bracts 12–18, ovate, deeply boat-shaped, spaced out, all reflexed, glabrous, bright red with a greenish-yellow margin, 3–4 in. long by 2 in. broad above the base. Flowers many in a cluster, yellowish, 2 in. long; bracts lanceolate, villose. Staminode small, spathulate. Peru, *Pavon*! *McLean*!

Subgenus **Stenochlamys**.

13. **H. dasyantha**, K. Koch et Bouché, Ind. Sem. Hort. Berol. 1854, app. 12; Regel, Gartenfl. t. 198; Peters. Fl. Bras. III, pt. 3, t. 3. Whole plant 5–6 ft. long. Leaves oblong, long-petioled, green and glabrous beneath, reaching a length of 2–3 ft., deltoid at the base. Panicle pendulous, from a long curved peduncle, above a foot long, 6–8 in. broad; rachis flexuose, very hairy; branch-bracts 7–8, lanceolate, shallowly boat-shaped, hairy outside, bright red with a green edge, the lower reflexed, 3–4 in. long, an inch round at the base and middle, the upper shorter, ascending. Flowers few in a cluster, hairy, yellow, 1½ in. long; bracts lanceolate, as long as the flowers. Staminode short, acute. Brazil, in woods at Maribi, *Martius*; French Guiana, *Leprieur*.

14. **H. platystachys**, Baker. Leaves very large, oblong, green and glabrous beneath, 3–4 ft. long, above a foot broad. Panicle

drooping from a short curved peduncle, which is densely clothed with soft bright brown hairs, deltoid, a foot long; rachis scarcely at all flexuose, clothed with hairs like those of the peduncle; branch-bracts few, lanceolate, spaced out on the rachis, arcuate-ascending, slightly pubescent on the back towards the base, the lowest 6–8 in. long, 1½–2 in. round above the shortly adnate base. Flowers few in a cluster, 2 in. long; pedicels finally an inch long, hairy; bracts lanceolate, as long as the flowers. Santa Marta, *Purdie*! Guatemala, alt. 5000 ft., *Donnell-Smith*, 1873! Intermediate between *dasyantha* and *latispatha*.

15. **H. brasiliensis**, Hook. Exot. Flora, tab. 190; Paxt. Mag. III, 193, with coloured figure; Kerner, Hort. t. 803. Whole plant 6–8 ft. long. Leaves long-petioled, oblong, green and glabrous beneath, 2–3 ft. long by 8–10 in. broad. Peduncle long, stiffly erect. Panicle deltoid, 6–9 in. long; rachis flexuose, pubescent; branch-bracts 8–12, lanceolate-acuminate, bright red to the edge, glabrous, the lower 6–9 in. long, 1–1½ in. round above the base, all arcuate-ascending. Flowers 6–9 in a cluster, dull green, or according to Petersen a handsome red or yellow, 2 in. long; ovary yellow; pedicels finally an inch long; tube very short. Staminode oblong. South Brazil, *Bowie* and *Cunningham*! *Burchell*, 1248! *Glaziou*, 8982! 18554! Banks of the Parana, *Parodi*! Amazon valley, *Traill*, 813! New Granada, *Triana*, 1647!

16. **H. latispatha**, Benth. Bot. Sulphur, 170 (1844); *H. meridensis*, Klotzsch, in Linn. XX, 462 (1847). Whole plant 6–8 ft. long. Leaves oblong, long-petioled, green and glabrous beneath, 2–3 ft. long by nearly a foot broad. Peduncle long, glabrous, erect. Panicle sometimes a foot long; rachis flexuose, nearly glabrous; branch-bracts 7–9, lanceolate-acuminate, all arcuate-ascending, the lowest 8–12 in. long, 1½ in. round at base, often leafy at the top, the upper gradually smaller. Flowers many in a cluster, 1½–2 in. long; pedicels pubescent. Staminode oblong-cuspidate. Andes of Bogota, *Hartwig*! Salango, Columbia, *Hinds*! Venezuela, *Moritz*, 1287! Panama, *Fendler*, 443! Guatemala, *Donnell-Smith*, 1829! A plant in Herb. Mus. Brit., gathered long ago by Shakespeare, is probably this species.

17. **H. lingulata**, Ruiz et Pav. Fl. Peruv. tab. 304. Whole plant 5–6 ft. long. Leaves oblong, long-petioled, green and glabrous beneath, obliquely cordate at the base, the lower 2–3 ft. long by nearly a foot broad. Peduncle erect. Panicle erect, nearly a foot long; rachis pubescent, but little flexuose; branch-bracts 12–20, all ascending, reddish-yellow, lanceolate, glabrous, the lowest 6–8 in. long, 1 inch broad at the base, ½ in. broad above the middle, not acuminate, as in *latispatha* and *brasiliensis*. Flowers many in a cluster, yellowish; pedicels ½–1 in. long. Staminode incurved, spathulate. Peru, *Pavon*! *Lechler*, 2679!
18. **H. Schiedeana**, Klotzsch, in Linn. XX, 463; *H. hirsuta*, Cham. et Schlecht. in Linn. VI, 57, non Linn. fil. Leaves long-petioled, oblong, green and glabrous except the pubescent mid-rib beneath and the pubescent petiole, 1–1½ ft. long, 6–8 in. broad. Peduncle and panicle erect; branch-bracts about 10, distant, lanceolate, pubescent, 2–3 in. long, under an inch broad; rachis pubescent, slightly flexuose. Flowers many in a cluster; bracts lanceolate; pedicels densely pubescent. Mexico, *Schiede*, 1031. Vera Cruz, Cordoba, &c., *Karwinski*.
19. **H. acuminata**, L. C. Rich. Nova Act. XV, Suppl. t. 11–12. Whole plant 6–8 ft. long. Leaves oblong, long-petioled, green and glabrous beneath, 1½–2 ft. long by 5–6 in. broad at the middle. Peduncle long, slender, stiffly erect. Panicle sometimes a foot long; rachis very flexuose; branch-bracts distant, lanceolate-acuminate, bright red, glabrous, the lowest 6–7 in. long, an inch round at the base. Flowers many in a cluster, 1½ in. long, reddish-green; pedicels finally an inch long. French Guiana, *Martin*! British Guiana, *Appun*, 257! *Jenman*, 905! 6373! Venezuela, *Fendler*, 1500! South Brazil, *Sello*! Amazon Valley, *Burchell*, 9860! A Demeraran plant from Jenman (473) with fewer and more distant branch-bracts, the lowest 2½–3 in. long is perhaps a variety. The species comes in half-way between *psittacorum* and *brasiliensis*. *H. Ballia*, L. C. Rich. in Act. Soc. Hist. Paris, I (1792) 107, is probably the same species, but the description is very incomplete. It is figured in Madame Mérian's Illustrations of the Metamorphoses of the Insects of Surinam, tab. 54.

20. **H. Burchellii**, Baker. Whole plant 6–8 ft. long. Leaves oblong, green and glabrous beneath, the upper shortly-petioled, the lower 1½–2 ft. long, 5 in. broad at the middle, rounded to the base. Peduncle long, glabrous, stiffly erect. Panicle subcernuous, ½ ft. long; rachis very flexuose; branch-bracts 7–8, rose-crimson, glabrous, all deflexed, lanceolate, the lower 2 in. long, ½ in. round at the base. Flowers many in a cluster, golden-yellow; pedicels pubescent, ½ in. long. Central Brazil; between Retiro and Rio Grande, *Burchell*, 5623! Differs from *acuminata* and *brasiliensis* by its very small deflexed branch-bracts.

21. **H. densiflora**, B. Verlot, in Rev. Hort. 1869, 274, with coloured figure. Whole plant 1½–2 ft. long. Leaves long-petioled, oblong, green and glabrous beneath, 4–5 in. broad, conspicuously cordate at the base. Peduncle long, slender, glabrous. Panicle dense, deltoid, ½ ft. long; rachis hidden, but little flexuose; branch-bracts 5–6, lanceolate-acuminate, glabrous, bright scarlet, the lowest 6–7 in. long, 1–1½ in. round at the base, the upper much smaller. Flowers 1½ in. long, bright yellow with a black tip. French Guiana, sent by Melinon to the Jardin des Plantes at Paris about 1869.

22. **H. hirsuta**, Linn. fil. Suppl. 158. Whole plant 3–4 ft. long. Leaves oblong, acute, green and glabrous beneath, without any petiole except the sheath which clasps the stem, ½–1 ft. long, 3–4 in. broad at the middle, rounded to the base. Peduncle long, erect, hairy. Panicle 3–4 in. long; rachis densely pubescent, flexuose; branch-bracts 6–8, arcuate-ascending, bright red, pubescent outside, lanceolate-acuminate, the lowest the largest, 3–4 in. long, under an inch broad. Flowers 6–12 in a cluster, bright yellow, pubescent outside, 1½ in. long. Staminode obovate-cuspidate.

    Var. **H. cannoidea**, L. C. Rich. in Nova Acta, XV, Suppl. tab. 9 and 10, fig. 2; Peters. in Mart. Fl. Bras. III, pt. 3, tab. 8; *H. vaginalis*, Benth. Bot. Sulphur, 171; *H. Richardiana*, Miquel, in Linn. XVIII, 70; *H. Swartziana*, Schult. Syst. Veg. V, 591; *H. psittacorum*, Brit. Mag. t. 502; *H. refracta*, Mart.; *H. bicolor*, Klotzsch, non Benth. Rachis, branch-bracts, pedicels and sepals nearly or quite glabrous. Throughout Tropical

America from Jamaica and Nicaragua to Peru and the South of Brazil.

23. **H. choconiana**, S. Wats. in Proc. Amer. Acad. XXIII, 284; Garden and Forest, 1888, 161, fig. 31. Whole plant 3–4 ft. long. Leaves without any free petiole, linear-oblong, green and glabrous beneath, 6–10 in. long, 2 in. broad, rounded to the base. Panicle subsessile, moderately dense, 3–4 in. long; branch-bracts 5–6, scarlet, glabrous, lanceolate-acuminate, the lowest long and leaf-pointed, the central 2–2½ in. long, not narrowed from the base to the middle. Flowers pale yellow, 2 in. long; pedicels glabrous, ½–¾ in. long. Staminode ovate, abruptly cuspidate. Guatemala; banks of the Chocon river, *Dr. S. Watson*.

24. **H. aurantiaca**, Ghiesb.; Lemaire, in Ill. Hort. tab. 332 (1862); *H. brevispatha*, Hook. in Bot. Mag. tab. 5416 (1863); *H. aurea*, Hort. Whole plant 2–3 ft. long. Leaves oblong, nearly sessile on the sheaths, green and glabrous beneath, the lower 9–12 in. long, 2–3 in. broad, broadly rounded at the base. Peduncle slender, erect, glabrous. Panicle erect, deltoid, 3–4 in. long; branch-bracts 3–4, lanceolate, erecto-patent, the lowest 3–4 in. long, an inch round at the base, orange-red with a green tip, the upper much shorter, entirely red-yellow; rachis but little flexuose. Flowers about 4 in a cluster, greenish-white, 2 in. long; pedicels short, red, glabrous. Staminode obliquely ovate. Forests of Southern Mexico. Introduced into cultivation by Ghiesbreght through Vershaffelt about 1860.

25. **H. angustifolia**, Hook. in Bot. Mag. tab. 4475; *H. bicolor*, Benth. in Maund Bot. tab. 101; Regel, Gartenfl. tab. 172; Horan. Prodr. Scit. tab. 4, non Klotzsch. Whole plant 3–4 ft. long. Leaves petioled, linear-oblong, very acute, cuneate at the base, green and glabrous beneath, the lower 1½–2 ft. long, 2½–3 in. broad. Peduncle erect, glabrous. Panicle deltoid, ½ ft. long; rachis but little flexuose; branch-bracts 6–7, lanceolate-acuminate, bright red to the edge, glabrous, the lowest 5–6 in. long, 1–1½ in round at the base, the others much shorter. Flowers 8–10 in a cluster, white, above 2 in. long; pedicels short, glabrous, orange-red. Staminode lanceolate. Brazil. Introduced into cultivation about 1846.

26. **H. psittacorum**, Linn. fil. Suppl. 198; Peters. in Mart. Fl. B
III, pt. 3, tab. 7, fig. 1; ***H. marantifolia***, G. Shaw. Wh
plant 2–3 ft. long. Leaves long-petioled, linear-lanceol
green and glabrous beneath, narrowed to the base, the lo
1–1½ ft. long, 1–2 in. broad at the middle. Peduncle lo
slender, glabrous. Panicle deltoid, 3–4 in. long; rachis
little flexuose; branch-bracts 4–5, lanceolate, erecto-pat
bright red, glabrous, the lower 3–4 in. long, under an i
round at the base. Flowers about 6 in a cluster, bright yell
with a black tip, 1½ in. long; pedicels ½ in. long. Stamin
tricuspidate.

Var. **H. Schomburgkiana**, Klotzsch, in Linn. XX, 4
H. psittacorum, var. *spathacea*, Eichl.; Peters. in Fl. Bras.
pt. 3, tab. 7, fig. 2. Taller and more robust, with broa
leaves more rounded at the base, and more numerous lar
branch-bracts.

Var. **H. subulata**, Ruiz et Pav. Fl. Peruv. tab. 3
H. angusta, Vell. Fl. Flum. III, t. 20; *H. Andrewsii*, Klotzs
in Linn. XX, 465 (Andr. Bot. Rep. tab. 124). Still m
robust, with leaves 3–3½ in. broad, panicle ½–1 ft. long w
a very flexuose rachis and lower branch-bracts 6–9 in. lo
an inch round the base. Throughout South America, fr
the West Indies to Peru and South Brazil.

27. **H. metallica**, Hook. in Bot. Mag. t. 5315. Whole plant 6–8
long. Leaves oblong, long-petioled, narrowed to the ba
bright green above, bright claret-purple all over benea
1½–2 ft. long, 4–5 in. broad at the middle. Peduncle slend
glabrous, stiffly erect. Panicle about ½ ft. long and broa
rachis but little flexuose; bracts distant, ascending, gre
glabrous, lanceolate-acuminate, the lowest much the larg
4–5 in. long, 2 in. round at the base. Flowers few in a clus
bright red with a greenish-white tip, 2 in. long; pedicels sh
Staminode ovate. Santa Marta, at the foot of the Sie
Nevada, *Schlim.* Introduced into cultivation about 18
H. vinosa, Bull Cat. 1871, 5 probably belongs here.

28. **H. pulverulenta**, Lindl. in Bot. Reg. t. 1048; Hook. in B
Mag. t. 4685. Whole plant 4–6 ft. long. Leaves lo
petioled, oblong, the upper 1–1½ ft. long, 4–5 in. broad, c
date at the base, white-farinose beneath. Peduncle lo

stiffly erect. Panicle deltoid, 6–8 in. long; rachis but little flexuose; branch-bracts about 5, lanceolate-acuminate, erecto-patent, glabrous, bright crimson, the lowest 6–9 in. long, ¾ in. round at the base, the others much shorter. Flowers many in a cluster, 1½ in. long, greenish-white; pedicels short. Stamino-de oblong, mucronate. West Indies, probably Dominica, *Hort. Kew.*! South Brazil, *Glaziou*, 18555! Trinidad, *Purdie*! *H. farinosa*, Raddi, and *H. dealbata*, Lodd., probably belong here. Of the former the leaf only is described, Mem. 4, Piant. Nuov. Bras. p. 14 (Modena, 1820).

29. **H. glauca**, Poit.; B. Verlot, in Rev. Hort. 1869, 112, with coloured figure. Whole plant 5–6 feet long. Leaves long-petioled, oblong, deltoid at the base, thinly white-farinose beneath, 1½–2 ft. long by 4–5 in. broad at the middle. Peduncle long, stiffly erect, glabrous. Panicle about ½ ft. long by 8–9 in. broad; rachis very flexuose, bright coral-red; branch-bracts about 5, distant, lanceolate, yellowish-green, glabrous, the lowest 5–6 in. long by an inch broad low down, the others shorter, the upper ascending, the central ones portent. Flowers 6–10 in a cluster, yellowish-green; pedicels and ovary bright red, the former ½–1 in. long. Demerara, *Drake*! Flowered at the Jardin des Plantes at Paris in 1869.

Genus 2. STRELITZIA, *Aiton.*

Flowers hermaphrodite. *Sepals* lanceolate, subequal, the one opposite the united petals more convex on the back. *Petals* very unequal, two connivent so as to form a saggittate blade in the centre of which is a channel that holds the stamens and style; the third very small, ovate. Perfect *Stamens* 5, as long as the petals; anthers long, linear, 2-celled. Ovary 3-celled; ovules many in a cell, superposed; style long, deeply divided at the apex into three branches. *Capsule* oblong, triquetrous, loculicidally 3-valved. *Seeds* few in a cell, furnished with a woolly arillus.—Acaulescent or caulescent. *Leaves* distichous, with a long petiole deeply channelled down the face and usually an oblong blade. *Peduncle* long or short; branch-bracts deeply boat-shaped, coriaceous, acute, usually single. *Sepals* bright yellow or white. *Petals* usually blue. The genus was named, at the suggestion of Sir Joseph Banks, after Charlotte, the

queen of George III, who was a princess of Mecklenburg-Strelitz.

Acaulescent. Sepals bright yellow . . . Sp. 1–2.
Caulescent. Sepals white . . . . Sp. 3–4.

1. **S. parvifolia**, (Dryand. in) Ait. Hort. Kew. ed. 2, II, 56; Bauer, Strelitz. tab. 11; *S. angustifolia*, Ait. loc. cit. Acaulescent. Petiole slender, reaching a length of 4–6 feet; blade oblong-lanceolate in the type, 8–9 in. long, 3 in. broad at the middle, deltoid at the base, bright green, with a narrow scariose brown edge, in *S. angustifolia* lanceolate. Peduncle about as long as the leaves, with green sheath-leaves; branch-bract 5–6 in. long, 2–3 in. round at the base, green with a reddish edge. Sepals bright orange-yellow, 3–4 in. long. Petals blue; lower with a blade about 2 in. long, with large round basal auricles. Stamens about 3 in. long.

   Var. **S. juncea**, Link, Enum. Hort. Berol. I, 15; *S. parvifolia*, var. *juncea*, Bot. Reg. t. 516; Reichb. Fl. Exot. t. 181. Blade of the leaf abortive. Cape Colony: south-western province, *Drège*! *Villette*! *Burchell*, 443873! Introduced into cultivation about 1796.

2. **S. Reginae**, (Banks, in) Ait. Hort. Kew. ed. 1, I, 285, tab. 2. Bauer, Strelitz. tab. 6–9; *S. regalis*, Salisb. Prodr. 145; *Heliconia Bihai*, J. Miller, Ic. tab. 5–6 (1780). Acaulescent. Petiole 3–4 feet long; blade in the type oblong-lanceolate, 1–1½ ft. long, 4 in. broad at the middle, cuneate at the base, crisped on the margin, especially downwards, bright green above, glaucescent beneath. Peduncle as long as the petiole; sheathing leaves green; branch-bract green with a reddish edge, 6–8 in. long, 2–3 in. round at the base. Sepals lanceolate, bright yellow, 3–4 in. long. Petals dark blue; blade of the two lower 1½–2 in. long, with a large round basal auricle; haft half as long as the blade; upper petal small, broad ovate. Stamens as long as the petals; anthers twice as long as the filaments. Style as long as the petals, its branches an inch long.

   Var. **S. glauca**, L. C. Rich. in Nova Acta, XV, Suppl. 17, tab. 2–3. Leaves oblong-lanceolate, glaucous. Peduncle not overtopping the leaves.

   Var. **S. farinosa**, (Dryand. in) Ait. Hort. Kew. ed. 2, II, 55. Petiole less than twice as long as the blade, which is oblong,

above a foot long, unequal sided and truncate at the base. Peduncle rather longer than the petiole, glaucous. Flowers exactly like those of the type.

Var. **S. ovata** (Dryand. in) Ait. Hort. Kew. ed. 2, II, 55; *S. Reginae*, Curt. Bot. Mag. t. 119-120; Andr. Bot. Rep. t. 432. Petiole and leaf-blade shorter than in the type, the latter rounded or subcordate at the base. Peduncle overtopping the leaves.

Var. **S. humilis**, Link, Enum. Hort. Berol. I, 150; *S. pumila*, Hort. A dwarf form, with petiole twice as long as the ovate concave blade and scape as long as the petiole. Cape Colony; south-western district, *Masson*! *Nelson*! *Burchell*, 3670! *Drège*! *Dr. Gill*! Rocky heights of Uitenhage and district of Albany near the Cowie river, *Bowie*! *S. prolifera*, Rafarin, in Rev. Hort. 1869, 159, fig. 13, is a form with two clusters of flowers; *S. Lemoinieri*, Meillez, in Flore des Serres, tab. 2370, a form with flowers more brightly coloured than usual and *rutilans*, Morren, in Ann. Gand, II (1846), tab. 53, a form with very dark orange-coloured sepals and leaves with a bright brown mid-rib and margin. Introduced into cultivation in 1773. See Miss North's drawings, No 365.

3. **S. augusta**[1], Thunb. Nov. Gen. 113; Prodr. 45; Bauer, Strelitz. tabs. 1–4; Bot. Mag. t. 4167; Flore des Serres, t. 173–4; *S. alba*, Spreng. *Heliconia alba*, Linn. fil. Suppl. 157. Caulescent when developed, with a short cylindrical trunk. Petiole reaching a length of 5-6 feet; blade oblong, bright green, deltoid or rounded at the base, reaching a length of 3–4 feet and a breadth of 1½–2 feet. Peduncle much shorter than the petiole. Branch-bract coriaceous, glaucous, claret-red, 8–12 in. long by 3–4 in. round at the base. Sepals lanceolate, white, 5–6 in. long. Petals also white, the two lower with the blades about 3 in. long with small rounded auricles and hafts half as long, dilated gradually towards the base; back petal ovate, an inch long. Stamens as long as the petals; anthers 3 in. long, twice as long as the filaments. Style-cusps 1½–2 in. long. Cape Colony, *Thunberg*, *Drège*; Durban, *Cooper*, 1225; Natal (I presume introduced). See Miss North's drawings,

[1] The name is misprinted *angusta* by Dietrich and others.

Nos. 359, 369, 374, *Krauss*! *Cooper*, 1225! Introduced into cultivation by Masson in 1791; yields a coarse fibre. There is a specimen from Masson's garden, with two clusters of flowers, at South Kensington.

4. **S. Nicolai**, Regel and Korn. in Gartenfl. t. 235; Flore des Serres, XIII, 121, tab. 1356; Hook. fil. in Bot. Mag. t. 7038. Whole plant reaching a height of 25 feet. Leaves like those of *S. augusta*. Branch-bracts a foot long, red-brown, 4–6 in. round at the base. Sepals white, lanceolate, 6–7 in. long, an inch broad. Petals blue, the blade of the lower 3–4 in. long, with a large ovate auricle; back petal orbicular, with a large cusp. Anthers 3–4 in. long, twice as long as the filaments. Style-cusps above 2 in. long. Cape Colony. No exact station is known. It is first known as having been seen in a garden in Madeira in 1849. It flowered at St. Petersburgh in 1858, and was named in compliment to the Emperor Nicholas.

Genus 3. RAVENALA, *Adans.*

(*Urania*, Schreb.)

Flowers hermaphrodite. *Sepals* 3, free to the base, lanceolate, convex on the back. *Petals* 3, free, lanceolate, one shorter. Fertile *stamens* 5 or 6, as long as the petals; anthers linear, 2-celled, basifixed. *Ovary* 3-celled; ovules many, superposed; style long, filiform, 6-cleft at the stigmatose apex. *Capsule* oblong-trigonous, coriaceous, loculicidally 3-valved. *Seeds* oblong, with a lacerated arillus.—*Trees* with a naked trunk. *Leaves* large, oblong, with a long petiole, dilated into a broad sheath at the base. *Panicle* with many spreading boat-shaped branch-bracts. *Flowers* white, many in a cluster in the axils of the bracts. *Fruit* the size and shape of a small banana, but rigid and not eatable.

Subgenus **Urania**.—Perfect stamens 6.

Subgenus **Phenakospermum**.—Perfect stamens 5.

1. **R. madagascariensis**, Sonner. Voy. Ind. II, 223, tabs. 124–126; Jacq. Hort. Schoen. tab. 93; Flore des Serres, tab. 1355; Belg. Hort. IX, 31; *Urania speciosa*, Willd.; *U. Ravenala*, L. C. Rich. Nova Act. XV, Suppl. tabs. 4–5. A tree, with a tall naked cylindrical trunk. Leaves 20–30 in a fan-shaped close distichous rosette; petiole very stout, reaching a length of

10–12 feet, with a wide-clasping base, often above a foot round; blade oblong, nearly as long as the petiole, 2 ft. broad. Panicles axillary, much shorter than the petioles, often more than one to a tuft; peduncle short; branch-bracts ovate, acute, coriaceous, deeply boat-shaped, 3–4 in. round at the base, spreading or the lower deflexed. Flowers white, 6–8 in. long. Perfect stamens 6; anthers 3–4 in. long. Stigma shortly lobed. Capsule 3–4 in. long, above an inch in diameter. Seeds in two rows, umbilicate, with a blue pulpy arillus. Madagascar. 'Commonly along the east coast, from sea-level up to 2000 feet, but also found occasionally, small in size, in the forests on the east side of Imerina, from 4000 to 5000 feet above sea-level. Besides good drinking-water, procurable from the base of its petioles, the leaves are used for thatching houses; their midribs, transfixed by long fine twigs, form house-walls and doors; the bark, beaten out flat, forms flooring, and the leaves also are used as plates and spoons. It usually grows in damp soil and always near water. The names are *Ravin-àla* (forest-leaves), *Akondro-àla* (forest banana), and *Akondro-hazy* (tree-like banana), because its height in crowded forests is from 90 to 100 feet.' Dr. *G. W. Parker*, F.L.S. It is frequently planted in tropical Asia and is included in Wallich's great Indian herbarium. Is in Miss North's drawings, Nos. 58, 535, 543.

2. **R. guianensis**, Benth. et Hook. fil. Gen. Plant. III, 657; Peters. in Mart. Fl. Bras. III, pt. 3, tab. 1, fig 3; *Urania guianensis*, L. C. Rich. in Nova Acta, XV, Suppl. tabs. 6–8; *U. amazonica*, Mart.; *Phenakospermum guianense*, Endl.; Miquel, Stirp. Surinam. 213, tabs. 62–63. Naked trunk reaching a height of 20–30 feet. Petiole much shorter than in the other species; blade oblong, 2 feet broad. Panicle with long peduncle 3–6 feet long, overtopping the leaves; branch-bracts 6–8, deeply boat-shaped, spreading, from 1–1½ ft. long. Flowers white; sepals 5–6 in. long; petals about an inch shorter. Perfect stamens 5, as long as the petals; anthers about 2 in. long. Stigma deeply cleft. Seeds in more than two rows; arillus when young yellow. French Guiana, *Sagot*, 578! Dutch Guiana, *Wullschlaegel*. Amazon valley near Para, *Burchell*, 9691!

Genus 4. MUSA, *Linn.*

Flowers unisexual, only those of the lower clusters producing fruit. *Calyx* at first tubular, soon slit down one side, 3–5-toothed at the apex. *Petal* placed opposite the calyx, simple or tricuspidate. Perfect *Stamens* 5; filaments filiform; anthers 2-celled, basifixed; rudiment of sixth stamen present or absent. *Ovary* cylindrical, 3-celled: ovules many in a cell, superposed; style filiform from a thickened base; stigma shortly lobed. *Fruit* indehiscent, pulpy or dry, oblong or cylindrical. *Seeds* subglobose or angled by pressure, often excavated at the hilum; testa hard, intruded at the base and apex; albumen farinaceous; embryo subtruncate.—Monocarpic shrubs with cylindrical or bottle-shaped trunks, often stoloniferous at the base. *Leaves* large, oblong, entire; free petiole long or short. *Panicle* of many clusters of flowers, spaced out on the rachis and each subtended by a large spathaceous scariose bract. *Flowers* usually white or yellow.

Key to the sections and species.

Subgenus **Physocaulis**. Stem bottle-shaped. Flowers many to a bract. Petal usually tricuspidate. Fruit not edible.

Tropical African . . . . . Sp. 1–5.
Indian . . . . . Sp. 6–7.

Subgenus **Eumusa**. Stem cylindrical. Flowers many to a bract. Petal ovate-acuminate. Bracts green, brown, or dull violet. Fruit usually edible.

Dwarf . . . . . Sp. 8–10.
Tall (group of *M. sapientum*) . . . . Sp. 11–21.

Subgenus **Rhodochlamys**. Stem cylindrical. Flowers few to a bract. Petal linear. Fruit usually not edible. Bracts bright-coloured, often red . . . . . Sp. 22–32.

Subgenus **Physocaulis**.

1. **M. Ensete**, Gmel. Syst. Nat. II, 567; Hook. in Bot. Mag. t. 5223–4; Rev. Hort. 1861, 124; Flore des Serres, t. 1418; Gard. Chron. 1881, 434, fig. 84; Miss North's drawings, No. 516; *Ensete edule*, Horan. Prodr. 40. Whole plant

30–40 ft. high. Stem ventricose at the base, non-stoloniferous. Leaves oblong, acute, bright green, reaching a length of 20 ft. and a breadth of 3 ft.; petiole short, broad, deeply channelled. Peduncle short; flowering panicle globose; bracts densely imbricated, ovate, 9–12 in. long, dark claret-brown. Flowers whitish, 1½–2 in. long, arranged in two rows, up to 20 in a row. Ovary cylindrical, above an inch long; calyx lingulate, 3-lobed at the apex; petal short, tricuspidate, with a large linear central cusp. Sixth stamen rudimentary. Fruit coriaceous, dry, 2–3 in. long. Seeds 1–4, black, glossy, transversely oblong, nearly an inch broad, with a prominent raised border round the hilum. Mountains of Abyssinia, southward to hills south of the Victoria Nyanza Lake. Niam-Niam land, *Schweinfurth*! Native name, *Ensete*. The pith of the young stems is much used as food by the Gallas and other tribes; also the young heads. For a full account see Bruce's Travels in Abyssinia, vol. vii. p. 149 (figured in his Atlas, tab. 89); Grant, in Trans. Linn. Soc. XXIX, 153, and Duchartre, in Sagot's Monograph, pp. 5–9. Grant's plant from Waganda, with a stem like two great drums placed one upon another, and Heuglin's from Semen, with stolons, will likely prove distinct species. It is the most hardy of all the cultivated species, growing freely in the open air in the Mediterranean region, and flowering freely at Kew in a cool conservatory (Temperate House). The seeds are commonly made into necklaces.

2. **M. ventricosa,** Welw. Apont. 585, No. 45. Not stoloniferous. Whole plant 8–10 ft. long. Stem much swollen, 4 ft. diam. at the base. Leaves oblanceolate-oblong, acute, bright green, 4–5 ft. long, much thicker in texture than in *M. sapientum*, with a pale red midrib; petiole very short and stout. Panicle drooping, dense, oblong-lanceolate, nearly as long as the leaves; peduncle very short and stout; bracts at the base of the spike 1–1½ ft. long, lanceolate; those of the fertile clusters oblong, 8–9 in. long, about 3 in. broad; flowers about 15 to a cluster. Fertile flowers 2 in. long; ovary cylindrical, under an inch long. Calyx 3-lobed, longer than the ovary. Petal ovate, entire, not tricuspidate, ⅛ in. long. Fruit like that of *M. Ensete*. Seeds as large as those of *M. Ensete*, dull black, with a broad hollow at the hilum. Angola; province of Pungo Andongo,

in rocky places near rivulets, *Welwitsch*, 6447! Differs from all the other species of this section by its entire petals. *M. africana*, Bull. Cat. 1871, 6, is probably this species in a young state.

3. **M. Buchanani**, Baker. Nearly allied to *M. Ensete*, but the bracts linear-oblong, in Buchanan's specimens 1–1½ ft. long, 2½–4 in. broad. Flowers 10 in a row. Ovary cylindrical, above an inch long. Unexpanded calyx cylindrical, as long as the ovary. Seeds as large as those of *M. Ensete*, glossy, black, not tubercled. Shiré highlands, *Buchanan*, 47, of 1885 collection! Sir John Kirk saw the seeds from the Shiré valley, at a height of 2000 ft. above sea-level.
4. **M. Livingstoniana**, Kirk, in Journ. Linn. Soc. IX, 128. Stem conical, twice the height of a man, 2–3 ft. diam. at the base. Leaves narrow oblong, crowded, as long as the trunk, with a short broad-clasping deeply channelled petiole. Fruit many-seeded, 4 in. long. Seeds globose, angled by pressure in the lower half, ⅛ in. diameter, dull brown, tubercled, with a depressed hilum, surrounded by prominent edges. South-east Tropical Africa from 12° to 19° south latitude, ascending to 7000 ft. Known to us only from Sir John Kirk's sketches and notes, and seeds which he brought home. There is a necklace of similar seeds in the Kew Museum, brought by Barter from Sierra Leone.
5. **M. proboscidea**, Oliver, in Hook. Ic. t. 1777. Not stoloniferous. Trunk dilated at the base, reaching 4–5 times the height of a man. Leaves narrow oblong, very large, 3–4 times as long as broad, narrowed to the base; free petiole, short, deeply channelled. Panicle-rachis finally drooping, very much elongated, nearly as long as the trunk; bracts broad ovate, obtuse, about 4 times as long as the flowers; flowers in two close rows of about 12 in a row. Calyx as long as the cylindrical ovary; petal very short, with two orbicular outer lobes and a large linear central cusp. Seeds turbinate, black, glossy, ½ in. long and broad, with only a small hollow at the hilum. Hills of Ukami, about 100 miles inland from Zanzibar. Known only from seeds and four photographs procured by Sir John Kirk.
6. **M. superba**, Roxb. Hort. Beng. 19; Corom. Plants, t. 223; Wright, Ic. t. 2017; Graham, in Bot. Mag. t. 3489–3450; Kerner,

Hort. t. 674. Whole plant reaching a height of 10–12 ft. Trunk much dilated, 7–8 ft. in circumference at the base, narrowed to 3 ft. below the leaves. Leaves oblong, narrowed to the base, bright green on both sides, 5 ft. long, 1½ ft. broad; free petiole very short, deeply channelled. Panicle at first globose, a foot in diameter, finally drooping, a third the length of the trunk; bracts orbicular, dull claret-brown, reaching a foot in length and breadth; flowers in two dense rows of 10–15 each. Ovary white, cylindrical, above an inch long. Calyx whitish, as long as the ovary, formed of three loosely cohering linear segments. Petal short, tricuspidate, with a large linear central cusp. Fruit oblong, subcoriaceous, 3 in. long, 1½ in. diam. Seeds very numerous, subglobose, angled by pressure, ⅓–½ in. diam., smooth, brown. Western Ghauts of the Bombay peninsula. Frequent in cultivation: introduced by Dr. J. Anderson to the Calcutta Botanic Garden in 1800. Yields a poor fibre.

7. **M. nepalensis**, Wall. in Roxb. Fl. Ind. edit. Wall. and Carey, II, 492. Trunk short, ovoid, 2 ft. in diameter at the base. Leaves rather smaller than in *M. superba*, and somewhat glaucous. Panicle at first dense, a foot in diameter, finally short, drooping; bracts dull purple, ovate, the lower ½ foot long; flowers 7–8 in a row. Calyx, petal, fruit and seeds like those of *M. superba*. Lower hills of Nepal, in dense shaded forests, *Wallich*. Described principally from two large unpublished drawings of Wallich, now at Kew. Not known in cultivation.

Subgenus **Eumusa**.

8. **M. lasiocarpa**, Franchet, in Journ. de Bot. 1889, 329, with figure. Whole plant 1–2 ft. long. Stems sending out at the base a stout horizontal rhizome. Leaves oblong-lanceolate, about a foot long, very glaucous, narrowed at the base to a petiole which is rather shorter than the blade, the broad truncate bases of the old leaves persisting round the base of the stem. Panicle dense, erect, oblong, under a foot long; bracts thin, yellowish, persistent, the upper ovate, the lower ovate-lanceolate. Flowers 4–8 in a cluster, above an inch long. Calyx 5-lobed. Petal shorter, ovate-oblong. Fruit oblong-trigonous, dry, pubescent,

with 4–6 seeds in each cell, which fill up the whole cavity. Mountains of Yunnan, alt. 4000 ft., *Delavay*. Franchet founds on this curious species a section called *Musella*, characterized by its membranous bracts and by possessing a rhizome.

9. **M. Cavendishii**, Lamb. in Paxt. Mag. III, 51, with coloured figure; Garden, 1891, II, 263; *M. chinensis*, Sweet, Hort. Brit. ed. 2, 596 (name only); Miss North's drawings, Nos. 225, 816; *M. sinensis*, Sagot. Stoloniferous. Whole plant 4–6 ft. high. Stem 2–3 ft. long, 3–4 in. diam. Leaves 6–8 in a dense rosette, spreading, oblong, 2–3 ft. long, about a foot broad, much rounded at the base, rather glaucous; petiole short, stout, deeply channelled, with two broad crisped green edges. Peduncle short, stout. Panicle dense, oblong, 1–2 ft. long, drooping; bracts red-brown or dark brown, ovate, the lower half a foot long, the upper 3–4 in.; male flowers and their bracts persistent. Calyx yellowish-white, an inch long, with 5 rounded lobes. Petal ovate, entire, less than half as long. Fruits as many as 200–250 to a panicle, oblong, 6-angled, slightly curved, 4–5 in. long, above 1½ in. diam., obtuse, narrowed gradually to the sessile base, seedless, edible, with a rather thick skin and delicate fragrant flesh. Seeds not known. Southern China. Introduced into cultivation by Telfair from Mauritius in 1829. The wild seed-bearing form is not yet known. *M. Massoni*, Sagot *Musa* 21 (name only), supposed to be wild at the Gaboon and cultivated in Bourbon, is said to be like *Cavendishii*, but with slightly different fruits.

10. **M. nana**, Lour. Fl. Cochinch. 644. Trunk cylindrical, 5 ft. long, ½ ft. diam. Leaves oblong-ovate, 3 ft. long. Panicle short, recurved. Flowers all fertile. Stamens often 6 or more. Fruit ovate-oblong, edible, seedless. Cochin China, *Loureiro*. Unknown to M. Pierre. It may be a form *M. Cavendishii*, Lamb., with a taller stem and staminate flowers abortive. I know nothing also of *M. Rhinozerotis*, Kurz, in Journ. Agric.-Hort. Soc. Ind. V, 64[1], which is said to be like *M. nana*, but to have the sheaths of all the leaves enveloping one another, persistent bracts and flowers all fertile.

11. **M. glauca**, Roxb. Hort. Beng. 19; Corom. Pl. t. 300. Not

[1] The elaborate paper of Kurz, above cited, was unfortunately cut short by his death, so that the full descriptions of his new species never appeared.

stoloniferous. Trunk cylindrical, 10–12 ft. long, 6–8 in. diam. Leaves oblong-lanceolate, acute, 4–5 ft. long, pale and glaucous, shortly petioled. Panicle drooping from the base; bracts greenish, persistent, the upper ovate, the lower ovate-lanceolate. Flowers 10–20 to a bract. Calyx whitish, about an inch long; segments 3, loosely coherent, linear. Petal small, tricuspidate, with a large linear central cusp. Fruit oblong, 4–5 in. long, 1½ in. diam., truncate at the apex, narrowed gradually to the sessile base. Seeds smooth, globose, nearly black, ½ in. diam. Pegu; introduced to the Calcutta botanical garden by Mr. F. Carey in 1810. This has flowers like *M. superba*, and a cylindrical trunk like *M. sapientum*.

12. **M. discolor**, Horan. Prodr. 41; Vieill. in Ann. Sc. Nat. 1861, 46. Stoloniferous. Stem slender, cylindrical, 6–10 ft. long. Leaves narrow-oblong, smaller and firmer in texture than in *M. sapientum*, rounded at the base, glaucous, tinged with violet or red beneath when young; petiole a foot or more long. Panicle drooping, finally as long as the leaves; bracts reddish, the upper only persisting; male flowers deciduous. Fruit cylindrical, angled, rather curved, umbonate at the apex, rather dry, reddish-violet, very palatable, with a violet pulp, with a rather musky scent. Wild in New Caledonia, according to Vieillard (native name *Colabonte*), and yielding textile fibre, which is used for fish-baskets, &c. It is widely spread in cultivation, and we have a drawing at Kew by Fitch of a plant that flowered there many years ago.

13. **M. Basjoo**, Sieb. et Zucc. (name); Baker, in Bot. Mag. t. 7182; *M. japonica*, Hort. Stoloniferous. Stem cylindrical, 6–9 ft. long, 6–8 in. diam. Leaves oblong, thin, bright green, 6–9 ft. long, 1½–2 ft. broad, deltoid at the base; petiole stout, about a foot long. Peduncle stout, arcuate, a foot long. Panicle dense, 1–1½ ft. long; female clusters 3–4, close, of 12–15 flowers each; bracts oblong, dull brown, the lower 8–12 in. long; male clusters 8–12, their bracts much imbricated, persistent. Calyx whitish, 2 in. long, shortly 5-toothed at the tip. Petal ovate-acuminate, nearly as long as the calyx. Fruit oblong-trigonous, 3 in. long, umbonate at the apex, narrowed gradually to the sessile base. Liu-Kiu archipelago, and cultivated in Southern Japan. Described from a plant that flowered

in the Temperate House at Kew in 1891. It is as hardy as *M. Ensete*, and is grown in Southern Japan for its fibre. *M. Martini*, Rev. Hort. Belg. 1892, 109, fig. 12, has the habit of *M. sapientum*, and is said to be more hardy than *M. Ensete*, with bright rose-red flowers. The leaves are oblong, long-petioled, firm in texture, bright green above, glaucous beneath, with reddish veins. It was brought from the Canary Islands.

14. **M. textilis**, Née. Ann. Cienc. IV, 123; *M. mindanensis* (Rumph. Amboin. V, 139); Miquel, Fl. Ned. Bat. III, 588; *M. sylvestris*, Colla, Monogr. Musa, 58; *M. Troglodytarum textoria*, Blanco, Fl. Filip. 247, ed. II, 173. Stem cylindrical, green, 20 ft. or more long, stoloniferous from the base. Leaves oblong, deltoid at the base, bright green above, rather glaucous beneath, smaller and firmer in texture than those of *M. sapientum*; petiole a foot long. Panicle drooping, shorter than the leaves; male flowers deciduous; bracts firmer in texture than those of *M. sapientum*, naked and polished outside, not at all pruinose, brown. Female flowers in several laxly-disposed clusters. Fruit green, oblong-trigonous, curved, 2–3 in. long, 1 in. diam., not narrowed to the apex, but narrowed to the short stout pedicel, not edible, but filled with seed. Seeds black, turbinate, $\frac{1}{6}$ in. diam., angled by pressure.

Var. **M. amboinensis** (Rumph. Amboin. V, 139); Miquel, loc. cit. Stem not so tall. Panicle not so drooping. Fruit as long as a man's finger, black at maturity. The type (*Vidal*, 3943 !) plentiful in the Philippine Islands, where it is called *Abaca*, and ascends the mountains to the lower limit of *Pinus insularis*, and is largely used in the manufacture of Manilla hemp, for information about which reference may be made to the Kew Bulletin for April, 1887. It is the most important of all cordage fibres, and the annual export from the Philippines to Britain is 170,000 bales, and to the United States 160,000 bales, equal to about 50,000 tons per annum. It was introduced into cultivation in India in 1811 by Dr. Fleming. As cultivated in the Peradeniya Botanic Garden it has thinner leaves more rounded at the base than the wild plant. Var. *amboinensis* in Amboyna.

15. **M. sapientum**, Linn. Sp. Plant. 1477; Trew, Ehret. t. 21–23; Rheede, Hort. Malabar. I, 17, t. 12–14; *M. sativa* seu *domes-*

tica, Rumph. Amboin. V, 130, t. 60; *M. paradisiaca*, Van Hooten, Fleurs Java, t. 30. Stem cylindrical, usually green, reaching a length of 20–25 ft., 4–6 in. diam., stoloniferous from the base. Leaves oblong, thin, bright green, 5–8 ft. long, 1½–2 ft. broad, usually rounded at the base; petiole 1–1½ ft. long. Panicle drooping, often 4–5 ft. long; male flowers deciduous; bracts lanceolate or oblong-lanceolate, dull violet, more or less glaucous outside, the lower 1–1½ ft. long, the upper ½ ft., often red inside, several expanded at once, the edges of the upper not involute. Flowers about a dozen to a cluster, yellowish-white, 1½ in. long; calyx 5-toothed at the top; petal ovate, half as long as the calyx. Fruits oblong-trigonous, 3–4 in. long, 1½–2 diam., forming 3 or 4 bundles of a dozen each, rounded to the apex, narrowed gradually to the sessile base, bright yellow when ripe, the flesh fit to eat without cooking. Universally cultivated throughout the tropical zone of both hemispheres for the sake of its fruit, and yielding also a fibre, which is much inferior in tenacity to that of *M. textilis*. The following are its subspecies and principal varieties, to which Latin names have been given, viz.:—

Var. **M. violacea**, Hort. Stem, fruit, and also often leaves beneath more or less tinged with violet.

Var. **M. sanguinea**, Welw. Leaves and fruit strongly tinged with blood-red.

Var. **M. odorata**, Lour. Fl. Cochinch. 644. Fruit delicate and fragrant.

Var. **M. mensaria**, Rumph. Amboin. V, 131. Fruit very palatable, subglobose, as large as an apple; flesh soft yellow; skin pealing away easily. Malay name *Pissang Medji*. Ripens early and soon decays.

Var. **M. regia**, Rumph. Amboin. V, 131. Fruit as long as a man's finger, an inch thick, very sweet and delicate in taste. Malay name *Pissang Radji*. Nearly allied to this is the *Gingeli* of Bourbon.

Var. **M. oleracea**, Vieill. in Ann. Sc. Nat. 1861, 46. A flowerless form, with a glaucous violet stem and an elongated thick turnip-like rhizome, which is boiled or roasted like a yam, which it resembles in taste. New Caledonia. Native name *Poiete*.

Var. **M. Champa**, Hort. Stem and midrib of the leaf red. Fruit pale straw-yellow, about 6 in. long, very luscious and delicate in flavour.

Var. **M. martabanica**, Hort. Fruit as in *Champa*, but midrib of leaf not red; border of petiole red-brown.

Var. **M. Dacca**, Horan. Prodr. 41. Stem pruinose. Leaves paler-geen than in the type, glaucous beneath; border of the petiole red. Fruit 4 in. long by half as broad, remaining tightly on the branch, its tip and stalk bright green; skin very thick. One of the common Indian forms.

Var. **M. rubra**, Firminger, non Wallich. Stem, petiole, flowers and midrib of leaf dull red. Fruit about 7 in. long, at first dark red, ripening to yellowish red. Indian name *Ram-Kela*.

Var. **vittata**, Hook. in Bot. Mag. t. 5402; *M. vittata*, Ackerm. in Flore des Serres, t. 1510–1513. Leaves and long fruits copiously striped with white. Spathes bright red inside. Imported from the island of St. Thomas, West Africa.

Subsp. 2. **M. paradisiaca**, Linn. Sp. Plant. 1477; Trew, Ehret. t. 18–20; Red Lil. t. 443–4; Tussac, Fl. Antill. t. 1–2; Rich. in Nova Acta, XV, Suppl. t. 1; *M. Cliffortiana*, Linn. Mus. Cliff. I, t. 1. Male flowers and bracts less deciduous. Fruit cylindrical, ½–1 ft. long, with firmer and less saccharine pulp, not fit to eat without cooking. Cultivated universally in the tropical zone.

Subsp. 3. **M. seminifera**, Lour. Fl. Cochinch. 644; *M. sapientum*, Roxb. Corom. Pl. t. 275; *M. sapientum* and *Troglodytarum*, Gaertn. Fruct. t. 11; *M. balbisiana*, Colla, Monogr. Musa, 56 (Rumph. Amboin. t. 60, fig. 3). Fruits small, oblong, full of seeds, not eatable, yellowish or greenish.

These names and figures apparently represent the wild seed-bearing form of *M. sapientum*, and if so it extends in a wild state from Behar and the Eastern Himalayas to the Philippine and Malay isles. The Chittagong plant figured by Roxburgh grows in very soft soil and has tall lanky stems. Kurz, in Journ. Afric. Hort. Soc. Ind. V, 164, distinguishes two species, *M. sapientum*, with spathes often crimson inside, seeds turbinate-globular to polyhedrous, tubercled, not above $\frac{1}{6}$ in. diam. and *M. sikkimensis*, with dull purple spathes and seeds de-

pressed and irregularly angled, tubercled, 4–5 lines diam. Of the latter we have careful sketches made on the spot by Sir J. D. Hooker and it has been widely distributed as *Musa* No. 5 of Hooker, and Thomson's Indian plants. Pierre, in Sagot's monograph, describes in detail three forms from Cochin China. *M. zebrina*, Flore des Serres, t. 1061–2, is, apparently, a dwarf form of this subspecies, with leaves copiously blotched with black.

Dr. King distinguishes four wild seminiferous forms in Sikkim as follows, viz.:—

1. *pruinosa* (*Reling* of the Lepchas). Stem 10–25 ft. long. Leaves very glaucous beneath, bracts deep violet purple, glaucous outside, red inside, persistent, subtending the fruit; fruit about 5 in. long by 1½ in. diam., permanently angled, seeds ¼ in. diam., pulp very scanty. Altitude 1500–3500 feet.

2. *dubia* (*Luxon* of the Lepchas). Stem shorter, leaves not glaucous beneath, bracts deep lurid purple not glaucous outside, purplish-red inside, lower bracts deciduous; fruit 3–4 in. long, 1–1⅓ in. diam. with 5–6 prominent ribs, seeds ⅛–⅓ in. diam., pulp more copious. Altitude 1500–5500 feet.

3. *Hookeri* (*Tiang-moo-foo-goon* of the Lepchas). Stem 10–14 ft. long, tinged with red, leaves bright green on both sides, tinged with purple when young, bracts purple on both sides, glaucous outside, lower deciduous; fruits 5–6 in. long 2 in. diam., prominently angled; seeds 4–5 in. diam., pulp scanty. Common between 4500 and 5500 feet.

4. *Thomsoni* (*Kergel* of the Lepchas). Stem green, 12–15 ft. long, leaves glaucous only when young, conspicuously cuspidated at the apex, bracts ovate, outside with vertical streaks of yellow and purplish-brown, yellow inside; fruit 2½ in. long, ¾ in. diam. faintly ribbed; seeds few, black, soft, ⅛ in. diam. surrounded by copious sweet pulp. Does not rise above 1500 feet.

Dr. King thinks the two latter forms as likely to be distinct specifically from *sapientum*. His *Hookeri* is probably *M. sikkimensis*, Kurz.

Subsp. 4. **M. Troglodytarum**, Linn. Sp. Plant. 1478; *M. Uranoscopos*, Rumph. Amboin. V, 137, t. 61, fig. 2. Fruits

small, crowded on the erect axis of the panicle, obovoid-oblong or nearly round, reddish-yellow, containing rudimentary seeds. Flesh sweet, yellow. Wild in India, Ceylon and the Malay isles, the favourite food of elephants. The above names have often been applied to forms of other species than *sapientum*, with a similar habit, such as *M. Fehi*.

For fuller information about the cultivated Bananas reference may be made to Rumph. Amboin. V, 125–137; Blanco, Fl. Filip. p. 239–246; Firminger's Manual of Gardening in India, ed. 3, p. 177; Bojer's Hortus Mauritianus, p. 331; Sagot, in Journ. Soc. Nat. Hortic. France, pp. 238–285; and Kurz, in Journ. Agric.-Hort. Soc. Ind. N. S. V, pp. 112–163.

I know nothing definite about *M. arakanensis*, Ripley, in Proc. Agric.-Hort. Soc. Ind. X, 51, a form yielding excellent fruit and fibre of poor quality.

There are wild, seed-bearing Bananas in the Solomon Islands, *Guppy*! and Timor Laut, *H. O. Forbes*! for the exact determination of which fuller material is needed.

16. **M. acuminata**, Colla, Monogr. Musa, 66; *M. simiarum* (Rumph. Amboin. 138, tab. 61, fig. 1); Miquel, Fl. Ned. Bat. V, 589; Kurz, in Journ. Agric.-Hort. Soc. Ind. XIV, 297: *M. Rumphiana*, Kurz, in Journ. Agric.-Hort. Soc. Ind. V, 164. Stem long, cylindrical, stoloniferous at the base. Leaves oblong, 5–6 ft. long, glaucous beneath, deltoid at the base, firmer than those of *M. sapientum*; petiole 1–1½ ft. long, almost without any membranous edge. Panicle drooping, shorter than the leaves; male flowers deciduous; bracts lanceolate or oblong-lanceolate, violet, only one of those of the female flowers, opened at once and revolute, those of the male clusters involute at the edge. Calyx white or yellowish, 1–1¼ in. long; petal ovate-acuminate, nearly as long as the calyx. Fruits in 4–6 clusters of 10–12 each, oblong, rostrate, 2–4 in. long, 1–1½ in. diam.; skin not easily peeled off; flesh sweet. Seeds dull black, angled by pressure, ⅛ in. diam. Common in Java and the other Malay islands, extending eastward to New Guinea. Kurz, who has studied this species carefully on the spot, says that a large proportion of the Bananas which are cultivated in the Malay archipelago are derived from it and that its best varieties are superior to all

those derived from *M. sapientum*, in quality and delicacy. Typical *M. acuminata* is wild and has fruits full of seed. From this several seedless cultivated varieties are immediately derived, differing in the colour of the leaves and fruit. They all have the leaves glaucous beneath, and in one form the waxy bloom is so copious that torches are made from it. Var. *violacea*, Kurz, has stems, leaves and flowers more or less tinged with dark purple, and purple 3–5-angled fruits with a thick beak. Its native name is *Peesang teembaya* or *Peesang hoorang* (Copper, or crab plantain). Var. *culta*, Kurz, is larger in all its parts, with much larger whitish or yellowish flowers and longer cylindrical or angled yellow or greenish seedless fruits. Of this there are 48 distinguishable varieties, of which the most curious is the Duck Plantain (*Peesang moolook bebbek*), the fruit of which has a beak nearly as long as its body. There is a fine series of these forms dried by Kurz from the Buitenzorg garden in the Calcutta herbarium and I refer here *M. paradisiaca*, Zollinger, Pl. Jav. Exsic. No. 3530. Probably *M. Berterii*, Colla, Monogr. Musa, 57; *M. aphurica* (Rumph. Amboin. V, 138, tab. 61, fig. 3), Miquel, Fl. Ned. Bat. III, 589, which has green and leaf-like lower bracts and pale yellow ripe fruit a span long, is a variety of this species. I know nothing of *M. Karang*, Kurz, in Journ. Agric.-Hort. Soc. Ind. V, 164, of which the fruits are said to be angular, short, and thick-beaked, and the bracts yellow inside.

A plant collected in the Andaman Islands by Kurz, with long-stalked rostrate fruits full of seed not more than an inch long including the beak, ¼ in. diam. when dried, and two numbers of his Burmese collection (Pegu, Yomah, 3282, 3283), with distinctly rostrate fruits full of seed, 2–2½ in. long without any angles when ripe, may be forms of *M. acuminata*, but require further study in a living state.

17. **M. corniculata** (Rumph. Amboin. V, 130), Lour. Fl. Cochinch. 644; Kurz, in Journ. Agric.-Hort. Soc. Ind. V, 161, 166, tabs. 2–4. Stem cylindrical, 10–12 ft. long, as thick as the human thigh. Leaves oblong, green, 5–6 ft. long; petiole 1–1½ ft. long. Panicle drooping, only the 2–3, rarely 4 lower bracts and flower-whorls developed, the former oblong-lanceolate, a foot long. Calyx deeply 5-toothed. Petal ovate-

acuminate, nearly as long as the calyx. Fruit cylindrical, a foot or more long, 1½–2½ in. diam., narrowed gradually to the apex and sessile base, golden-yellow when ripe; skin thick; pulp reddish-white, firm, dry, sweet, very palatable when cooked. Malay isles and Cochin China. Kurz (loc. cit.) compares the fruit to a cucumber as regards shape and size and describes five varieties, but considers it to be probably only an extreme form of *M. acuminata*. A curious form is the Lubang variety, of which the stem is said to produce only a single fruit, large enough for a full meal for three men.

18. **M. Hillii**, F. Muell. Fragm. IX, 169, 190. Not stoloniferous. Stem cylindrical, very robust, reaching a height of 30 ft. and a diameter of 1½ ft. Leaves oblong, arcuate, bright green, similar to those of *M. sapientum* in colour and texture, reaching a length of 12–15 ft. and a breadth of 2 ft. Peduncle 3 in. diam. Panicle dense, erect; bracts oblong or oblong-lanceolate, 3–9 in. long. Flowers not numerous in a cluster. Calyx about an inch long. Fruits densely crowded, not edible, sessile, ovoid, much angled, 2–2½ in. long, umbonate or obtusely acuminate at the apex. Seeds numerous, angled, much depressed, ⅛–¼ in. diam., with a bony testa. Queensland: banks of the Daintree river, with the two other species, *Fitzalan*. We have a plant now in the Palm-house at Kew which has not yet flowered. In habit it resembles *M. Troglodytarum*, Linn. No doubt this is *M. Jackeyi*, Kurz, in Journ. Agric.-Hort. Soc. Ind. N. S. V, 64.

19. **M. Fitzalani**, F. Muell. Fragm. IX, 188. Stem cylindrical, 20 ft. long. Leaves patent, oblong, 10–12 ft. long by 2 ft. broad. Panicle drooping. Flowers 7–10 to a bract; upper bracts ovate or oblong, 2–3 in. long. Calyx nearly an inch long. Fruits oblong, angled, yellow when ripe, not pulpy, 2–3 in. long, narrowed suddenly to a thick pedicel about ½ in. long. Seeds numerous, filling the cells, angular, depressed, scarcely ¼ in. in diam. Queensland: banks of the Daintree river, *Fitzalan*. *M. Charlioi*, Walter Hill, in Report of the Brisbane Garden, 1874, is said to have stems 40–50 ft. long, leaves 5–6 ft. long, and fruits 3–4 in. long.

20. **M. Banksii**, F. Muell. Fragm. IV, 132; Benth. Fl. Austral. VI, 261; *M. Banksiana*, Kurz, in Journ. Agric.-Hort. Soc. Ind.

N. S. V, 64. Stoloniferous, with a cylindrical trunk, like that of *M. sapientum*. Leaves oblong, 5–6 ft. long, 1½–2 ft. broad, bright green; petiole 1½–2 ft. long. Panicle drooping; upper bracts oblong, 3–4 in. long, lower much longer. Flowers 10–20 to a bract. Calyx 1–1¼ in. long, shortly 5-lobed; outer lobes lanceolate, inner shorter, oblong. Petal ovate-lanceolate, ½ in. long. Fruits quite cylindrical when dry, without any angle, straight, coriaceous, under an inch in diameter, obtuse at the apex, narrowed suddenly to a slender stipe 1½–2 in. long. Seeds grey, subglobose, ⅛ in. diam., angled in the lower half. Queensland: Mount Elliot, Rockingham Bay, &c., *Herb. F. Mueller*! Very like *sapientum* in stem and leaf, but totally different in fruit. It yields a fibre of poor quality.

21. **M. Fehi** (Bertero), Vieill. in Ann. Sc. Nat. 1861, 46; *M. Fei*, Nadeaud, Fl. Tahiti (1873), 39. Stoloniferous. Trunk cylindrical, 15–20 ft. long, greenish, full of violet juice. Leaves larger and firmer in texture than in *M. sapientum* and *paradisiaca*, with stouter veins; midrib green; base unequally rounded; petiole 1–1½ ft. long. Panicle long, erect, slightly curved only at the base. Flowers 6–8 in a cluster, sessile. Calyx with 5 unequal lobes, split finally nearly to the base. Petal short. Fruits many in a bunch, oblong, angled, 5–6 in. long by above an inch in diameter, nearly straight, yellow when ripe, with a thick skin and moderately firm pulp, not very palatable when raw, but excellent when cooked. Seeds small, dull black. Common in the forests of Tahiti, where it is largely used for food, seedless at the low levels, but bearing seeds at an altitude of 3000–3600 feet. Native name *Fei*. Found also sparingly by Vieillard in New Caledonia. Native name *Daak*. We have a young plant at the present time in the Kew collection. Probably the Fijian *M. Seemanni*, F. Muell. Fragm. IX, 190 (name only), of which a photograph, sent by Sir John Thurston, is reproduced Gard. Chron. 1890, II, 162, fig. 28, is the same species. This is *M. Uranoscopos*, Seem. Fl. Vit. 290, and *M. Troglodytarum*, Kurz, in Journ. Agric.-Hort. Soc. Ind. N. S. V, 163, in part. We have also leaves from the Rev. T. Powell of a plant from Samoa called *Laufoo* which probably belongs here.

Subgenus **Rhodochlamys.**

22. **M. maculata**, Jacq. Hort. Schoen. t. 446; Kerner, Hort. t. 667. Stem slender, cylindrical, 7–8 ft. long. Leaves oblong, obtuse, deltoid at the base, green above, glaucous beneath, 2½ ft. long, 6–8 in. broad; petiole ½ ft. long. Panicle drooping from above the base; male flowers deciduous; spathes yellowish-brown, the upper oblong, 3–4 in. long; flowers about 4 in a cluster. Calyx yellowish-white, above an inch long, 5-toothed at the apex: petal linear, obtuse, entire, nearly as long as the calyx. Fruit oblong, 2–3 in. long, 1 in. diam., narrowed gradually to the sessile base and apex, yellow, spotted with brown, eatable, aromatic; flesh, white. Known only as cultivated in Mauritius and Bourbon, where it is called *Figue mignonne*. Differs from the other species of this section by its eatable fruit.

23. **M. sumatrana**, Beccari, Cat. Hort. Flor. II, 4; André, in Ill. Hort. N. S. t. 375. Whole plant 7–8 ft. long. Stem slender, cylindrical. Leaves oblong, 5–6 ft. long, 1½ ft. broad, glaucous, with irregular blotches of claret-brown, rounded at the base; petiole slender, a foot long. Peduncle hairy. Panicle more or less drooping; male flowers deciduous; upper spathes small, orbicular, densely imbricated (colour not known); fertile portion consisting of about six clusters of four fruits each spaced out on a flexuose rachis above a foot long. Flowers an inch long. Calyx 5-toothed at the apex; petal linear, obtuse, nearly as long as the calyx. Dried fruits cylindrical, curved, 2–3 in. long, ½ in. diam., narrowed suddenly to a slender stipe ½–1 in. long. Sumatra; province of Padang, alt. 1100 feet, *Beccari*, 489! Our specimen in flower is from the Poona Botanic Garden, sent by Mr. G. M. Woodrow. Its affinity is evidently with *M. rosacea*, Jacq.

24. **M. rosacea**, Jacq. Fragm. t. 132, fig. 4; Hort. Schoen. t. 445; Bot. Reg. t. 706; Lodd. Bot. Cab. t. 615; *M. ornata*, Roxb. Hort. Beng. 19; Fl. Ind. I, 666; *M. speciosa*, Tenore; *M. Carolinae*, Sterler. Stoloniferous. Stem cylindrical, 3–5 ft. long, 3–4 in. diam. Leaves linear-oblong, 3 ft. long, under a foot broad, tinged with purple beneath; petiole long and slender. Panicle drooping or erect, finally a foot long; bracts ovate-lanceolate, pale blue or reddish-lilac, the lower 6–8 in.

long, the upper oblong, about 3 in. long, edges involute; female flowers in few clusters, 3–4 flowers in each; male clusters very numerous, most of the bracts falling. Calyx yellow, an inch long, 5-toothed at the apex; petal linear, obtuse, nearly as long as the calyx. Fruit oblong, obscurely 4–5-angled, yellowish-green when ripe, 2–3 in. long, but little pulpy, scarcely edible. Seeds ½ in. diam., black, tubercled, angled by pressure, rarely produced in the cultivated plant. Eastern Himalayas and hills of the Concan. It flowered at Kew in Oct. 1881 and June 1890, and we have a specimen collected in the hill-tracts of Chittagong by Mr. J. S. Gamble in Feb. 1880. It was introduced into Europe from Mauritius about 1805.

25. **M. salaccensis**, Zolling. Pl. Exsic. Jav. No. 1353; Kurz, in Journ. Agric.-Hort. Soc. Ind. XIV, 301. Stem slender, cylindrical. Leaves thin, oblong, green on both sides, 2 ft. long, 8–9 in. broad at the middle, cuneate at the base; petiole short. Panicle drooping, a foot long; nodes very numerous and crowded; flowers greenish, 2–3 to a cluster; bracts pale lilac, upper oblanceolate-oblong, obtuse, 2–3 in. long. Calyx above an inch long; petal linear, as long as the calyx. Fruit oblong, full of seed, 3 in. long, under 1 in. diam. when dried, narrowed gradually to a short stout pedicel. Seeds dull brown, angled by pressure, ½ in. diam. Mountains of Java and Sumatra. Described from specimens in the Calcutta herbarium, dried by Kurz from the Buitenzorg Garden. Nearly allied to *M. rosacea*.

26. **M. coccinea**, Andr. Bot. Rep. t. 47; Red. Lil. t. 307–8; Ker, in Bot. Mag. t. 1559; Peters. in Mart. Fl. Bras. III, pt. 3, t. 1; Van Hooten, Fleurs Java, t. 39; Miss North's drawings, No. 696; *M. Uranoscopos*, Lour. Fl. Cochinch. 645, excl. syn. Rumph. Stem stoloniferous, slender, finally 4–5 ft. long, 2–3 in. diam. Leaves oblong, 2–3 ft. long, 6–9 in. broad; petiole long, slender. Peduncle erect. Panicle dense, erect, finally half a foot long, with few clusters of female flowers with 3–4 flowers in each; bracts bright red or tipped with yellow, the lower lanceolate, ½ ft. long, the upper oblong, about 3 in. long. Flowers yellow, an inch or more long. Calyx 5-toothed at the tip; petal linear, obtuse, nearly as long as the calyx. Fruit

oblong-trigonous, yellow, not edible, 2 in. long. Seeds very small, oblong, rarely produced in cultivation. Southern China and Cochin-China. Introduced into cultivation in 1791, and now widely spread. It yields a fibre of poor quality.

27. **M. rosea**, Herb. Hort. Bot. Calcutt. Habit of *M. coccinea*, but leaves much shorter and broader in proportion to length, thin, green, about a foot long by half as broad, deltoid at the base and apex; petiole deeply channelled, nearly as long as the blade. Panicle short, erect; rachis pubescent, not flexuose; bracts pale red; lower lanceolate, half a foot long; upper oblong, obtuse, about 2 in. long; flowers 2–3 in a cluster. Calyx an inch long; petal as long as the calyx. Fruit and seeds not seen. Described from two specimens in the Calcutta Herbarium, dried from the Botanic Garden in June 1882.

28. **M. rubra**, Wall.; Kurz, in Journ. Agric.-Hort. Soc. Ind. XIV, 301. Habit of *M. coccinea*. Leaves oblong-lanceolate, 1½–2 ft. long, 6–9 in. broad at the middle, acute, deltoid at the base; petiole slender, a foot long. Peduncle and panicle erect, the latter at first dense, the fruiting part finally ½–1 ft. long; nodes very numerous and crowded; bracts bright red, glabrous; lower sterile, lanceolate, a foot long; upper oblong, 3–4 in. long. Calyx yellow, an inch long, 5-toothed at the tip; petal lanceolate, half as long as the calyx. Fruits in 3–4 clusters of 3–4 each, cylindrical, glabrous, dry, 1½–2 in. long, ½ in. diam., narrowed to the base in a distinct short stipe. Seeds smooth, dull brown, ¼ in. diam. Rangoon, *M'Clelland*! Yomah, Pegu, *Kurz*, 3282! 3283! Differs from *M. coccinea* by its short petal.

29. **M. sanguinea**, Hook. fil. in Bot. Mag. t. 5975. Stem very slender, 4–5 ft. long. Leaves oblong, 2–3 ft. long, thin, bright green, rounded at the base; petiole slender, a foot long. Panicle erect, or finally drooping; female clusters 2–6, with 2–3 flowers in each; male clusters few, dense; bracts bright red, the lower lanceolate, ½ ft. long, the upper persistent, lanceolate, 3–4 in.; rachis stout, pubescent. Calyx bright yellow, 5-toothed at the apex, 1½ in. long; petal linear, obtuse, nearly as long as the calyx. Fruit oblong-trigonous, 2 in. long, rather pulpy, pale yellow-green variegated with red, glabrous. Seeds angled by pressure, small, black, tubercled. Assam; Mahuni

forest, *Mann*! Introduced into cultivation in 1872. *M. assamica*, Hort. Bull is allied plants, at present imperfectly known, which may prove to be distinct.

30. **M. Mannii**, Wendl. MSS. Stem slender, cylindrical, 2 ft. long, 1 in. diam., tinged with black. Leaves few, spreading; petiole 6–10 in. long; blade oblong, green, unequally rounded at the base, 2–2½ ft. long, 9–10 in. broad. Peduncle with spike erect, ½ ft. long; female flowers in three clusters of three flowers each, their bracts deciduous; male bracts crowded, oblong, pale crimson, 3–4 in. long. Calyx pale yellow, 1½ in. long; petal much shorter, truncate. Assam. Described from a specimen that flowered in the palm-house at Kew, March 1893.

31. **M. velutina**, Wendl. and Drude, in Regel, Gartenfl. 1875, 65, t. 823; *M. dasycarpa*, Kurz, in Journ. Agric.-Hort. Soc. Ind. XIV, 381. Habit of *M. sanguinea*. Leaves oblong, unequal at the base, narrrowed into the long petiole. Panicle short, dense, erect; bracts bright red, pubescent on the outside; lowest sterile, lanceolate; upper oblong-lanceolate, 5–6 in. long. Fertile flowers about 3 clusters 3–4 in each; male clusters 6–9-flowered. Calyx pale yellow, 1–1½ in. long, 5-toothed at the apex; petal as long as the calyx, entire, obtuse. Fruit velvety, bright red. Throughout the forests of Assam, *Mann*. Introduced into cultivation in 1875. Differs from *sanguinea* and *aurantiaca* by its red pubescent fruit.

32. **M. aurantiaca**, Mann, Herb. Habit of *M. sanguinea*, but forming larger clumps of rather shorter stems. Panicle moderately dense, finally 8–9 in. long; rachis glabrous; bracts bright orange-yellow, glabrous; lowest sterile, lanceolate, a foot long; upper oblong-lanceolate, persistent, 3–4 in. long; female flowers in 4–5 clusters of 2–4 each. Calyx yellow, above an inch long, 5-toothed at the tip; petal linear, obtuse, as long as the calyx. Fruit green, glabrous. Forests of Upper Assam, *Mann*! Differs mainly from *M. sanguinea* by its orange-coloured bracts.

On Dischidia rafflesiana (Wall.)[1].

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With Plate X.

I. The Functions of the Pitchers.

Of the forty-six species which, according to Beccari[2], comprise the Asclepiadaceous genus *Dischidia*, most are confined to the Malayan region (including New Guinea, Ké Isles, Moluccas, and Philippines): a few occur in India: one in tropical Australia: one is peculiar to Hong-Kong, and another to Formosa. All are twining epiphytes; but, as far as is known, pitchers are possessed only by *D. rafflesiana*, *D. timorensis*, *D. complexa*, and the three doubtful species *D. merguiensis* (Becc.), *D. clavata* (Wall.), and *D. digitiformis* (Becc.).

D. rafflesiana twines round the trunks and branches of trees, and it appears to prefer decaying trees[3]. Whilst some of its leaves are normal foliage-leaves, others are converted into shortly-stalked pitchers. Each pitcher is oblong ovate

[1] See also preliminary communication in Proceedings of the Royal Society, vol. 53.

[2] Beccari, Malesia, vol. ii.

[3] Griffiths, Notulae, vol. iv.

and laterally compressed: the aperture or mouth is small and its lips are deeply incurved (see Fig. 1). The mouth is directed upwards or downwards, towards or away from the support. The plant climbs by means of adventitious roots which spread over the bark of the host-tree, and even dip beneath any loose portions of the bark. The stem, or the stalk of the pitcher, gives off a second class of roots which enter and ramify within the cavity of the pitcher.

Concerning the fact that *D. rafflesiana* is specially found on decaying trees, a few explanatory remarks may be ventured upon. Schimper [1] shows that the distribution of South American epiphytes is, within their own region, largely determined by: (1) their relative demands for light and moisture, some epiphytes requiring more light and a less constant supply of moisture than others; (2) the denseness or lightness of the foliage of the host-plant, including the deciduous or evergreen character of the latter; (3) the nature of the bark or surface of the host-plant, epiphytes for the most part avoiding trees with a very smooth surface or with a peeling bark; (4) the nature of the host, epiphytes showing a decided preference for certain trees in a manner at present not wholly explicable. In the case of a decaying tree the foliage will be less dense, consequently any epiphyte upon it will receive more light and a larger direct supply of rain-water, than if the tree were healthy. The air and the soil in the neighbourhood of this lightly-foliaged tree will be, on the average, drier, and the humus on its surface will be more rapidly decomposed. Thus, in a moist climate, an epiphyte on a decaying tree will have at its disposal a larger amount of available food than if it grew on a healthy tree of the same sort. This would seem to explain the fact mentioned by Schimper [2], that in the moist and equable climate of Jamaica *Aspidium nodosum* can live on decaying trees (not on healthy ones): whereas in Trinidad, which has a more marked dry

[1] Schimper, Die epiphytische Vegetation Amerikas (Botan. Mittheil. aus den Tropen, Heft 2, Jena).

[2] Schimper, loc. cit.

season, this plant is able only to live in the pockets, filled with moist humus, formed by the persistent leaf-sheaths of Palms. In regions not uniformly moist, the life of an epiphyte growing on a decaying tree will be endangered by the rapid evaporation of the water, and the speedy decomposition of the humus, on the surface of the host. *D. rafflesiana* is able to exist in spite of these dangers, because its stem and leaves possess a thick cuticle which is coated with wax: and, in addition, because its pitchers can store up water and nutritive material. Moreover, owing to the relative thinness of the leaf-canopy of its host, rain-water can pour directly into some of the pitchers. Whether or not *D. rafflesiana* actually requires a large amount of light, cannot be decided without further observation. But in its peculiar habitat on decaying trees—even when the bark is already peeling off—this plant escapes many epiphytic competitors which would struggle with it for food and light.

For our knowledge of the plant we are mainly indebted to Wallich [1], Griffiths [2], Beccari [3], and Treub [4]. Treub gives an account of previous observations and of the hypotheses advanced concerning the functions of the pitchers, as well as the results of his own careful investigations into the anatomy, development, and contents of the pitchers. He shows that, since the epidermis which lines the cavity of the pitcher is coated with wax, it is not adapted for the absorption of liquids [5]. Inside the pitchers he found living ants; frequently water; occasionally particles of detritus; and rarely a dead insect or so. He concludes, 'En somme le rôle principal, si

[1] Wallich, Pl. Asiat. Rar. vol. ii.

[2] Griffiths, loc. cit.

[3] Treub, Sur les urnes du *Dischidia rafflesiana* (Wall.).

[4] Beccari, loc. cit.

[5] It would be worth while to conduct experiments calculated to show to what extent liquids may be absorbed through the numerous stomata which are present in the epidermis lining the cavity of the pitcher. Each stoma, however, communicates with the cavity of the pitcher by the agency of a relatively long narrow canal traversing the wax which is raised into a small turret above each stoma. Hence surface tension, and the presence of air in these canals, would greatly hinder, or wholly check, the absorption of liquids during the life of the pitcher.

non unique, des urnes du *Dischidia rafflesiana*, est de recueillir ou, à un moindre degré, d'épargner de l'eau.' Beccari observes the same contents, but he lays stress on the occurrence of living Acari within the pitchers. He points out that the pitchers are specially adapted for the retention of water; and he mentions the occurrence in them of 'detritus' which would be of some use in adding to the store of available food. His startling conclusion as to the mode of origin and use of the pitchers, may be given in his own words: 'E ciò io l'attribuirei all' origine galloide degli ascidi, ereditari solo perchè la pianta, invece di risentirne danno, ha potuto utilizzarzli in vario modo, sopra tutto come organi protettori delle radici nei momenti di siccità.'

The present observations may be briefly described as complementary to those of Treub and Beccari: they are intended to fill up a gap which, owing to circumstances, exists in the observations of these two distinguished investigators. The work was commenced in May 1892, at Singapore, and the plants observed grew in the Botanic Garden there on a thinly-foliaged decaying tree.

To begin with *observations made on living plants at Singapore*, I cannot confirm the testimony of all previous observers as to the constant presence of considerable numbers of living ants in the pitchers; I saw but few ants. However Mr. Ridley has since (in December 1892) found them abundantly in pitchers of the plants I observed. I found living Acari, occasionally living chelifers and scale-insects in the pitchers.

In some cases dead and decaying insects, fungi living on organic matter, and insect excreta, were present but never in any considerable quantity. On the other hand, large quantities of earthy particles, including small stones, mingled with humus (fragments of leaves, &c.), were found in the pitchers. In many cases these substances choked the pitchers and even blocked up their mouths[1]. Finally, water was present in

[1] A figure is given (Fig. 1) of a pitcher taken haphazard from alcohol-material; it shows that there is a considerable amount of earthy matter left in the

a goodly number of pitchers; it frequently was rendered turbid by the amount of matter suspended in it.

In the last case it is obvious that the pitchers contained a nutrient liquid, but the question remains to what extent, if any, this nutrient solution can be utilized by the plant. In order to prove that the roots inside the pitchers can absorb liquid, I made the following experiments on living plants. Two tolerably young pitchers were separately removed from the plant and the pitcher-roots of one of them were severed. The two isolated pitchers were then carefully filled with water and hung up in open air. Both pitchers remained for some days apparently unchanged: then the pitcher with the severed roots, showed signs of shrivelling and blanched slightly. Unfortunately my experiments could not be continued longer than a week, but they serve to illustrate the fact that the pitcher-roots can absorb water. Other facts tend to show that the solids in the pitcher are utilized by the roots. In the first place I found that the pitcher-roots possessed root-hairs: and clinging fast to the latter were particles of earth which adhered just as do fragments of soil to the root-hairs of terrestrial plants (Fig. 10). I frequently noticed that in empty pitchers the root-hairs were excessively short. In the second place the root-system was well developed in every pitcher rich in the solid substances referred to, though the converse was not true. In pitchers containing a quantity of solid matter, water was frequently absent, though I cannot in every case state that there had never been any water in these pitchers. But water cannot have been the sole, or even the principal, agent in inducing the strong development of the roots of the pitchers alluded to. For I found that when the solid contents were specially collected on one side of the pitcher the roots were in a corresponding manner more largely developed on that side. This one-sided distribution of solid matter was not confined to horizontally-placed pitchers, so it could not have been occasioned by the disposition of rain-

cavity, although, doubtless, some must have been washed out during the voyage from Singapore.

water in the pitchers. This extensive development and copious branching of the root in a pitcher with solid contents can only be explained either on the basis of Nobbe's experiments proving that roots branch more abundantly in nutritious soils: or by supposing that, for some reason, the earthy particles had been conveyed to the regions in which the roots had been already more extensively developed. This latter alternative cannot at once be dismissed as absurd, as will be seen when the source of the particles is considered: but it is negatived by the observation that root-hairs are short in the empty pitchers, but large and persistent in the presence of the solid substances. Thus there can be no doubt that the pitcher-roots utilize the masses of earthy substance and organic matter found in the pitchers. Even when rain does not fall into the latter, the hygroscopic contents acquire water from the damp air and from the moisture which, as shown by Treub, collects even in pitchers which rain-water cannot reach; and is doubtless largely derived from the aqueous vapour transpired through the stomata of the epidermis lining the cavity of the pitcher.

At first sight it appears a matter of some difficulty for rain-water to get into pitchers unless their mouths are directed upwards and exposed to the rain, and I subsequently made some experiments on *alcohol-material* in order to see if rain-water can enter pitchers which are in some other position. I selected a not uncommon position of the pitchers for the purpose, i. e. the mouth of the pitcher was directed towards the supporting stem and pressed close against it; the position, in fact, which the pitchers would always assume did they retain their phylogenetically original arrangement. To produce the effect of a heavy tropical rain on a lightly-foliaged tree, water was poured from the rose of a watering-can directly on to the trunk or branch against which the pitcher was held. The pitcher chosen was one which had when living been naturally pressed against the bark of the host-tree to such an extent that it was flattened around the lips of the mouth. In the experiments the pitcher was held in its natural

position with its mouth in contact with the bark of a *Taxodium distichum*. The results were somewhat surprising when we consider the deeply incurved lips of the mouth. A considerable amount of water entered the pitcher held against an upright trunk, or against the inferior face or the side of a thick, gently sloping branch. No water entered when the pitcher was held against the upper side of the same branch. Thus rain-water can get into some pitchers even when rain cannot fall directly into them. In such cases as these it is clear that the pitcher-roots could utilize soluble matters in any solid contents which might be present in the pitchers. But there still remain to be accounted for those pitchers which are so placed that rain-water cannot enter them under any circumstances.

The question arises, Whence come the considerable quantities of earth and humus which the pitchers frequently contain? Till one makes experiments one is apt to very much underrate the mass of solid substance which, after even one heavy rainfall, is floated down the surface of a tree—especially of a tree with a partially disintegrated or peeling bark, such as that of the tree from which I collected my *Dischidia*-material. But making due allowance for this fact, I think that the solids in the pitchers examined were mainly brought by ants. Mr. H. N. Ridley, whose observations on ants' nests are well known, agrees with this view and states that they are the remains of ants' nests. Whilst at Singapore I noticed that these particles were present in pitchers which could not be reached by rain-water, and that they were sometimes arranged loosely throughout the pitcher-cavity, and at other times on one side only. Amongst the fragments of earth were small pieces of leaves which looked exactly as if they had been cut. Mr. Ridley has recently observed that the earth found in the pitchers is the same as that growing at the foot of the host-tree: he found clay in the pitchers and clay at the foot of the tree. All these facts harmonize with the view that the particles were largely brought by the ants, just as ants bring fragments of leaves, &c., to build nests under

the concave leaves of *D. Collyris* (Wall.). In the literature there appear to be no definite statements as to whether the ants merely utilize the pitchers as homes, or whether they bring material with which to build nests. Treub, however, says 'Les ascidies sont devenues de véritables nids de fourmis.'

The next question which requires an answer is, For what purpose did the pitchers originally arise? Treub has worked out the development of the pitchers; the ontogeny of the pitchers supports the view of the evolution which is obtained from a comparison with other species of *Dischidia*[1]. There are many species in which the leaves are concave on neither face; a form of *D. nummularia*, which I saw growing abundantly about Singapore, possesses small fleshy leaves which are biconvex in section and afford no shelter to the roots. *D. cochleata* (Bl.) has leaves with a convex upper surface and concave, pale purple, lower surface: the leaves in this case tend to diminish the loss of water by the roots. In *D. Collyris*, Wall. (*Conchophyllum*, Bl.) the densely set leaves, lying close over the surface of the host-plant, have concave purple lower surfaces, and they completely screen the roots; in the spaces under the leaves ants build nests and bring hither stores of humus (small fragments of leaves, &c.), for the purpose. Finally some of the leaves of *D. rafflesiana* have their lower surfaces so concave, and their margins curved into the concavity, to such an extent, that the pitcher-form is the result. In addition the roots in connexion with the pitchers altogether desert the surface of the host and ramify within the cavities of the pitchers. There is little reason to doubt that originally the concave leaves protected the underlying roots, as they do in *D. Collyris*: and as do the flattened pseudo-bulbs of the epiphytic *Oncidium Limminghii*[2] and the flattened stem of the epiphytic *Polypodium schomburgkianum*[2].

But with regard to the final stage in the evolution of the pitchers, five views may be propounded. It may be suggested that, having attained the concave form now seen in *D. Collyris*,

[1] See Beccari, loc. cit.

[2] Goebel, Pflanzenbiologische Schilderungen, i, pp. 228-229.

they further developed into pitchers which should serve as (1) traps in which to catch insects; (2) dwelling-houses for ants which should in return protect the plants from the attacks of animals; (3) root-protectors; (4) water-reservoirs; (5) receptacles for food-material (humus, &c.). Naturally two or more of these functions might be combined.

Treub has dismissed the trap-theory because, in the first place, the corpses of insects are found in the pitchers in extremely small quantities; secondly the insects found in the pitchers are for the most part living and they can easily escape; thirdly there are no digestive glands within the pitchers. However it may be pointed out that, after all, the difference between the mode of action of the pitchers of *Dischidia* and of those of the indubitably insectivorous *Nepenthes*, is not so wide. Insects can and do escape from the pitchers of *Nepenthes*: they are regular nurseries for the larvae of mosquitos which develop in the contained liquid [1] and subsequently fly out. I have myself seen a spider rush out from beneath the incurved margin of the pitcher of *N. ampullacea*. *Nepenthes* is by no means dependent on animals for its humus; I examined scores of pitchers of *N. ampullacea* and found that most of the open pitchers contained decaying vegetable matter in the form of whole leaves or fragments of them. And, finally to break down the supposed widest difference between the pitchers of *Dischidia* and *Nepenthes*, it now appears that in *Nepenthes* they possess no ferment-secreting digestive glands, but that the fermentative processes are the work of Bacteria contained in the liquid within the pitchers [2]. Nevertheless the preponderance of living insects within the pitchers of *Dischidia*, and

[1] H. N. Ridley, Proceedings of the Asiatic Society (reprint, not dated). I have confirmed Ridley's observations and have found that the mosquito-larvae not only develop in the liquid, but that the mature mosquitos escape. I plugged isolated pitchers with cotton-wool and suspended them on a verandah at Singapore; on subsequently removing the plugs many mosquitos flew out.

[2] R. Dubois, Sur le prétendu pouvoir digestif du liquide de l'urne des *Nepenthes*. Comptes rendus de l'Académie des Sciences de Paris, Tome cxi, 1890, p. 315. Dubois' work is not conclusive, but his views appear to be corroborated by the

the insignificant amount of dead insects there, sufficiently show that the pitchers did not arise as traps for insects.

As regards the view that the pitchers arose merely as quarters for a standing army of ants, there is nothing to support it.

The view that the pitchers were evolved solely as organs for the protection of the roots, cannot be taken by itself: for it is obvious that the roots within the pitchers would be of no use unless the latter contained substances which the roots could absorb.

Treub adheres to the theory that the pitchers arose for the purpose of storing up water. In favour of this view one can cite the analogy of other epiphytes which possess water-reservoirs (aqueous tissue of leaves, pseudo-bulbs of orchids, the thickened petioles of some Aroids[1]). But on the other hand, the fact is that the position of the pitchers is not the most perfectly calculated for them to merely catch rain-water. With this may be coupled the occurrence of a considerable amount of solid matter in the pitchers. In the stage of evolution of the pitchers in which they were merely leaves with concave lower surfaces, the main or sole supply of water to the underlying roots would be—not pure rain-water but—water running down the trunk and branches of the host and carrying with it solid particles. Hence one could easily conceive that the pitchers might have been evolved in order that this nutritive liquid should be stored up and made available for the roots. And it is quite possible that this is the whole *rationale* of the origin of the pitchers.

Some facts, however, seem to indicate that the attainment of the pitcher-form has been associated with the storage of food brought by the ants: (1) Ants live under the leaves of other species of *Dischidia*, namely under leaves with concave lower surfaces: (2) *D. Collyris* has leaves which are more like

fact that mosquito-larvae develop within the liquid of the pitchers and by Tischutkin (Ueber die Rolle der Mikro-organismen bei der Ernährung der insektenfressenden Pflanzen. Ref. in Botan. Centralbl. L. 1892, p. 304).

[1] Schimper, loc. cit.

the pitchers of *D. rafflesiana* than those of any other species not possessing pitchers—that is they are at a stage of evolution immediately preceding the pitcher-stage—and ants build nests under these leaves, bringing thither fragments of leaves, &c.: (3) Ants are constantly found in the pitchers in all the widely distant spots, in the Malayan region, at which the plant has been observed[1]: (4) The pitcher-form ensures that seclusion from light which ants love when nesting; (5) it has already been mentioned that probably the greater part of the solid matter found in the pitchers is conveyed thither by ants.

The one objection to this view is that, as rain-water can get into some of the pitchers, the existence of the ants is imperilled. But the truth is that ants constantly nest in much more dangerous positions as far as the risk of inundation is concerned. Wallich certainly found large black ants drowned in the pitchers, but observations of other observers show that this is an exceptional occurrence. It is no argument against the view to say that, as ants are so abundant in the tropics, it would be curious if they were absent from the pitchers. The very fact of the prevalence of ants in tropical regions, makes it possible for them to be an important factor in the evolution of the surrounding flora.

It has been pointed out that the lips of the pitcher are sharply incurved. If a pitcher be filled with water and then reversed, only a little water escapes: in fact the pitcher is roughly constructed on the principle of the common ink-pot from which ink cannot be spilt. And this may be the rationale of the use of the incurved margins. But the latter would also provide a platform for the ants when the mouth is directed downwards, and a shade when the mouth points upwards. However ants prefer the shade when nesting, and hence would possibly show a preference for pitchers the mouths of which were not directed upwards, especially as in such pitchers there is less danger of their being swamped.

[1] Singapore, Malacca, Borneo, Java, Billiton. It would be an extremely valuable piece of information if it could be ascertained if the same species of ant is always present.

Imbued with the idea that ants may be the source of a considerable revenue to the plants, an enquiry into the literature of myrmecophilous plants naturally suggests itself. Have we evidence that any other epiphytes are directly benefited by the presence of ants? or do any myrmecophilous plants give off adventitious roots in an abnormal manner in order to utilize supplies brought by ants? Neither of these questions can be certainly answered. However with reference to the former question it may be pointed out that Schomburgk states that he found ants constantly present amongst the roots of certain epiphytic species of *Epidendrum* and *Coryanthes*: he adds that the ants were so numerous that it was impossible to collect the flowers without being bitten by them. Appun[1] says that these orchids perish if the ants are removed from them. Still there is no evidence that ants bring stores of humus to the roots of these plants, though such is possibly the case. With regard to the second question, ants are constantly found living in immense numbers in the galleries which excavate the swollen, tuber-like, bases of the stems of *Myrmecodia* and *Hydnophytum*. In both these genera adventitious roots are developed from the walls of the galleries. Beccari figures a copious formation of adventitious roots within the galleries of *Hydnophytum guppyanum*[2] (Becc.). Guppy observed that a dirty liquid was contained in the central chamber of this system of galleries. Beccari points out that similar adventitious roots are not known to be developed to such an extent in other species of *Hydnophytum* or *Myrmecodia*: so he associates these two facts and supposes that the roots are developed for the purpose of absorbing the store of food thus laid before them. But Beccari has not microscopically examined these supposed roots. Treub[3] found in the galleries of a Javan *Myrmecodia* lenticels and a few feeble roots. He shows that the lenticels are not normal, but he decides that they do not absorb liquids. Of the roots he states

[1] Quoted in Goebel, loc. cit.

[2] Beccari, loc. cit.

[3] Treub, Sur le *Myrmecodia echinata*, Ann. Jard. Bot. Buitenzorg, vol. ii.

that they are feebly developed. But one might suggest that, as the development of roots in the pitchers of *Dischidia* is increased by increased supply of solid food, and as Treub's observations on *Myrmecodia* were made mostly on plants deserted by the ants peculiar to the plant, possibly in normal plants there might have been more vigorous roots. It is highly probable that ants do bring substances into the galleries of *Myrmecodia* and *Hydnophytum*, and it is extremely suggestive that within these galleries occupied by ants are lenticels through which water may be transpired and thus moisture be supplied to any solids present, and that there are also roots which can absorb any nutriment available. So in the pitchers of *Dischidia rafflesiana* there are stomata through which moisture may be supplied to the solids in the pitcher, and there are also roots by which the nutrient liquid may be absorbed.

To sum up:

(1) The pitchers are by no means mere water-reservoirs, though they often contain water which is of use to the plant. They are depositories for solids such as earth, humus, &c., from which, by means of the roots within the pitchers, the plant derives an essential portion of its nutriment.

(2) The solids in the pitchers are partly derived from detritus washed down the stem and branches of the host-plant by the rain; but they are also, and perhaps chiefly, brought by ants which nest in the pitchers.

(3) The phylogeny of the pitchered leaf, traced through those species which have merely concave leaves, seems to indicate the evolution of an organ more perfectly adapted to provide shelter for the ants, on the one hand, and on the other to secure for the use of the plant the materials collected by the ants. Side by side with this main factor, the formation of pitchers conferred upon the plant the power of storing up rain-water and substances brought down with it.

II. The Structure of the Roots.

Dischidia rafflesiana is by no means peculiar in possessing

roots of two sorts. Schimper[1], in his masterly work on epiphytes, points out that there are two classes of epiphytes which possess roots of two kinds. First there are those which have negatively heliotropic roots, which are simply organs of attachment: and other roots which are organs of absorption, the latter being positively geotropic and descending to the ground. Secondly there are epiphytes which have negatively heliotropic roots which, though primarily organs of attachment, are to a certain extent absorptive: and, in addition, other roots which are negatively geotropic and purely absorptive. *D. rafflesiana* forms a third class of heterorhizal epiphytes; for it possesses attaching-roots which are also absorptive, as well as the pitcher-roots which are purely absorptive but possess no marked geotropic properties[2]. Neither is it a unique phenomenon that the absorptive roots should be associated with peculiar humus-collecting leaves, for the same is the case with heterophyllous epiphytic Platyceriums. The analogy between these Ferns and *D. rafflesiana* is rendered still more striking from the fact, discovered by Goebel[3], that ants nest amongst the humus collected in the mantle-like leaves of *Platycerium alcicorne*.

As the external conditions of the two sets of roots of *D. rafflesiana* are dissimilar—one set creeping over the shoots of the host and the other set hidden from direct sunlight—and, as the pitcher-roots have no attaching function, we should expect to find corresponding differences in structure. And our anticipations become all the stronger by reason of the fact that other epiphytes display anatomical distinctions in their two sets of roots[4]. The pitcher-roots do differ in structure from the attaching-roots, but the differences are not

[1] Schimper, loc. cit.

[2] The pitcher-roots appear to be negatively heliotropic, but no experiments have been made showing that this is the case.

[3] Goebel, loc. cit. It is not known whether the ants simply utilize the particles of vegetable matter, &c., contained in the niches formed by the mantle-leaves, or whether they take thither stores of vegetable fragments, earth, &c. In the latter case the resemblance to the state of affairs in *D. rafflesiana* would be very close.

[4] See Schimper, loc. cit.

so fundamental as to prevent the two being described together.

In the young stage the root is clothed externally by a layer of epidermal cells, many of which grow out into root-hairs. In the attaching-roots these hairs are mainly developed on the side of the root which is in contact with the surface of the host-plant ('ventral side'): elsewhere the root-hairs are much less numerous and are smaller. On the ventral side they form a dense mycelium-like weft, the marginal root-hairs of which are longer, whilst the central ones are very short (Figs. 2, 9). Each root-hair, on reaching the bark of the host, bends and runs for some distance over its surface. In the younger parts of some attaching-roots there is a curious grouped arrangement of the ventral hairs; when the root is examined from above, one sees that in each cluster of hairs there is a gradual increase in the length of the marginal hairs as we travel away from the apex, and then there is a diminution in their length: then follows a space with a few dwarfed hairs which are in turn succeeded by another cluster similar to that described. Whether this curious grouping be due to differences in the degree of contact, or to irregularities in the mode of distribution of moisture or nutritive matters on the surface of the bark, I cannot say. It seems probable that the formation of numerous and large root-hairs is not solely a phenomenon due to contact. For, in the first place, on pitcher-roots the hairs are longer and larger on the side towards the solid contents. In the second place, in one case it was observed that an attaching-root spread over some Mosses clothing the trunk of the supporting tree: and though the root was separated from the moss-leaves by an appreciable distance, yet, on the side facing them, it developed numerous large hairs which spanned the intervening space and finally spread over the surface of these leaves. In the third place, long hairs are occasionally developed on the sides, and even the dorsal face, of an attaching-root: and, in this case, they are directed parallel to the supporting surface, or are even obliquely inclined towards it. The latter two facts

suggest that the mode of development and arrangement of the root-hairs is partially dependent on the local distribution of moisture. The pitcher-roots are, for the most part, not in contact with the walls of the pitcher, hence the epidermis and root-hairs are uniformly developed round the root (except where the contents of the pitcher are unevenly distributed): but where these roots come into contact with one another, or with the wall of the pitcher, there is a formation of hairs similar to that of the attaching-roots excepting that there are no long marginal hairs. Hence the latter are peculiar to the attaching-roots, and certainly they are admirably adapted to hold the root fast to the supporting surface. The hairs of the pitcher-roots, and the ventral hairs of the attaching-roots, persist and live for a long time, but they become cuticularized tolerably early in life. In the attaching-roots the process of cuticularization takes place at an earlier date, and is more extensive, than in the pitcher-roots. Thus the walls of these hairs are well cuticularized whilst the lateral walls of the epidermal cells to which they belong still consist of pure cellulose.

Except at the region of contact, the epidermis of the root speedily disintegrates and the subepidermal cells form the external coat of the root. They form a distinct layer of cells with cuticularized and lignified walls, and have no intercellular spaces between them: in fact, they constitute a typical epidermoidal layer (exodermis). Two sorts of cells take part in the formation of this layer: first, ordinary cells, elongated in the direction of the long axis of the root, and possessing only a thin film of protoplasm lining the walls which are by no means thick; secondly, smaller cells, of the same radial diameter as the preceding, but not elongated in the direction of the long axis of the root, possessing a very thick suberized external wall, and having a conspicuous nucleus embedded in a considerable mass of protoplasm. These two varieties of cells form longitudinal lines of cells which often regularly alternate in such a manner that the small cells almost look as if they had been cut off from the ends of the long cells.

Each of the small cells (Figs. 2, 3, 4, 5, 6, 7, 8) is roughly conical in shape, with a base arched outwards and a truncate apex inwards. Its cell-wall is completely cuticularized and lignified. In a tolerably young stage the outer wall shows the following structure: the lamellae of the epidermal cell (still consisting of cellulose) are succeeded internally by a thick, cuticularized and lignified membrane which is traversed by radial lines (Fig. 5). A surface view shows that, corresponding to these fine lines, there are round dots: so that this view of the outer wall reminds one of a sieve-plate with minute pits (Fig. 6). Hence the wall is traversed either by fine canaliculi, or by rods of substance different to that constituting the rest of the wall. I was unable to decide which of these two suggestions is correct. In addition there is very often a large pit in this outer wall (Fig. 7). The question arises as to the function of these conical cells. In shape, arrangement, and abundant protoplasmic contents, they correspond to the passage-cells found in epidermoidal layers. But, in place of having thin cellulose-walls, they have very thick heavily suberized outer walls. However, for the following reasons, it is probable that water can pass through these walls. First, there is often a large pit in the external wall. Secondly, the radial striation of this wall is especially marked in younger cells, i.e. in cells which occupy regions still capable of absorbing liquids; whilst in older parts which have ceased to absorb, the striation is often replaced by a granulated appearance. Thirdly, at the region of contact with the supporting bark the epidermoidal cells are smaller than elsewhere, but the diminution of size takes place particularly in the elongated cells: hence in this region the surface of the conical cells is relatively increased, and this is precisely the spot at which absorption of liquids is most active. So there is ample reason to conclude that the conical cells are peculiar passage-cells, and probably that the striae in their external walls denote channels for the conduction of liquids. In some of these conical cells various bodies occurred, in the shape of spore-like granules, bacteroid-like bodies, and large stratified masses

attached to the cell-wall. Owing to their comparatively infrequent occurrence (at any rate in my material) I could not determine the nature of these bodies; all of them were faintly stained yellow with iodine but refused to stain with Hofmann's blue. Gram's method for staining Bacteria was employed, and results were obtained which at first appeared to show that Bacteria were present within these cells (Fig. 8). But use of Zeiss' $\frac{1}{12}$ oil immersion with an Abbe's condenser, disclosed the fact that the peculiar staining effects were only produced in ectoplasmic regions: it also showed that the rough method of fixing the roots (by immersing the plants in methylated spirit) had induced local irregularities and aggregations in the protoplasm. Hence it is highly probable that the peculiar stained portions were only dense pieces of ectoplasm.

Within the epidermoidal layer lies the rest of the cortex. In the attaching-roots this consists solely, or mainly, of thin-walled parenchymatous cells which are distinctly smaller on the ventral side of the root. Occasionally two or three feeble strands of sclerenchyma occur close within the epidermoidal layer of the dorsal side. Thus there is a distinct dorsiventral structure in the cortex of these roots (Fig. 9). In the pitcher-roots, which, for the most part, hang free from the wall of the pitcher, the cortex is marked by the presence of strong bands of sclerenchyma usually separated from the epidermoidal layer by one layer of cells (Figs. 10, 5, 3). The radial symmetry of the cortex of the pitcher-roots is but slightly disturbed when the root is in contact with the wall of the pitcher, in that a band of sclerenchyma appears never to occur immediately within the centre of the region of contact. Starch and crystals of calcic oxalate are found in the cortical parenchyma.

Olivier[1] has pointed out that, in aerial roots, the development of cork is precocious; and *D. rafflesiana* forms no exception to this rule. The cortical layer beneath the epidermoidal layer becomes a phellogen, and periderm is formed

[1] Olivier, Recherches sur l'appareil tégumentaire des racines. Ann. des Sci. Nat. 6, xi, 1881.

towards the exterior. But the two classes of roots differ in the finer details in the mode of formation of cork. The dorsiventral structure of the attaching-roots is further accentuated by the fact that cork is not formed within the immediate region of contact (Fig. 9); and this is also true when contact is only ensured by the development of long root-hairs, as in the case quoted of the root spreading over a Moss. The object of this arrangement is obvious: the formation of cork on the side in contact would check the absorption of nutrient solutions. In pitcher-roots cork appears to be formed only in their older parts, near the mouth of the pitcher, i.e. in regions where the root is most likely to become dry and where there would probably be no liquid to absorb. Here it is developed round the whole circumference, and secondary patches of phellogen originate within the bands of sclerenchyma.

The vascular cylinder displays no general differences in the two varieties of roots, except that in some attaching-roots dorsiventral structure again appears (Fig. 11). In such a case, on the ventral side of the central mass of xylem, a few wood-vessels appear which greatly exceed all the rest of the wood-elements in the size of their lumina. That is, there is a group of larger vessels on the side on which the absorption of water is taking place. In this case the large size of these vessels cannot be attributed to differences of pressure; if any difference of pressure existed at the time when the vessels were forming it would decidedly be greater on the ventral side, as can be seen from the tangential flattening of the young cortical cells which takes place on that side alone. These large wood-vessels are probably the result of a demand for more ready conduction of water on the side where absorption of liquids is taking place.

In conclusion, I desire to express my thanks to Professor Vines for affording me the hospitality of the Botanical Laboratory of the University of Oxford, thus greatly facilitating the work involved in the foregoing investigation.

EXPLANATION OF FIGURES IN PLATE X.

Illustrating Mr. Groom's paper on *Dischidia rafflesiana.*

rh, root-hairs. *ep*, epidermis. *ex*, epidermoidal layer or exodermis. *p*, passage-cells of the epidermoidal layer. *sc*, sclerenchyma. *ck*, cork. *ph. b*, bundles of phloëm in the central cylinder of the root. *s*, bark of the supporting tree. *V*, side towards the support. *D*, side away from the support.

Fig. 1. A pitcher (from alcohol-material) laid open longitudinally, showing the one-sided development of the roots, and the solid contents still remaining in the pitcher.

Fig. 2. Transverse section of the region of contact of an attaching-root, showing the root-hairs. (Zeiss 3 D. subsequently reduced to one-half.)

Fig. 3. Semi-diagrammatic longitudinal section through the outer layers of a pitcher-root. (Magn. Zeiss 3 D. subsequently reduced to one-half.)

Fig. 4. Surface section through the inner part of the epidermoidal layer. (Zeiss 3 D.) This figure explains how it is that in transverse section on each side of a passage-cell there is apparently a small narrow cell; really the two apparent cells are the processes of one large cell which partially embraces the passage-cells.

Fig. 5. Transverse section of part of a pitcher-root, showing the striation of the external wall of a passage-cell.

Fig. 6. Surface view of the external wall of a passage-cell.

Fig. 7. Section showing pits in the external walls of two passage-cells. (Zeiss $\frac{1}{12}$ oil immers. oc. 4.)

Fig. 8. A passage-cell in longitudinal section stained with Gram's method for staining Bacteria. (Zeiss $\frac{1}{12}$ oil. immers. oc. 8.)

Fig. 9. Transverse section through an attaching-root, showing dorsiventral structure.

Fig. 10. Transverse section through a pitcher-root showing unilateral development of the root-hairs, and particles clinging to the latter (alcohol-material.)

Fig. 11. Transverse section of the central vascular cylinder of an attaching-root, showing the dorsiventral structure of the central mass of xylem.

Fig. 1.

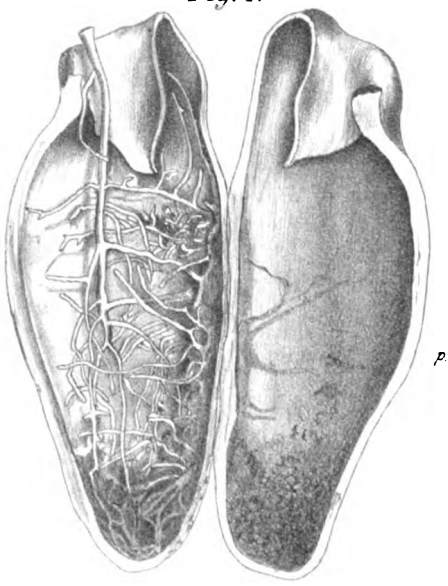

Fig. 4.

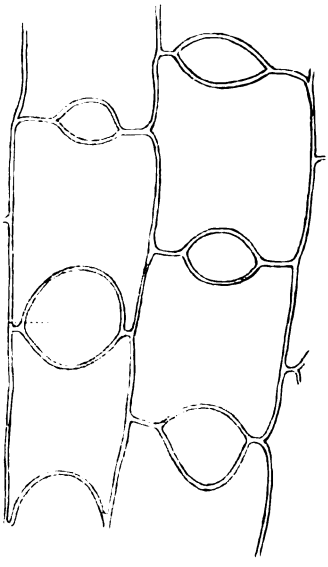

Fig. 3.

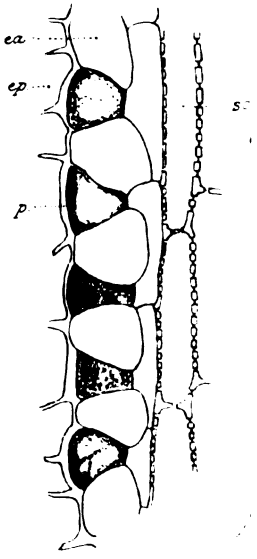

Fig. 6.

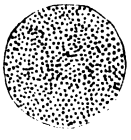

Fig. 2.

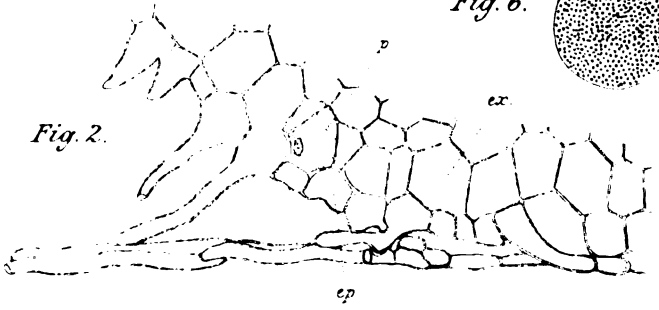

Fig. 8.

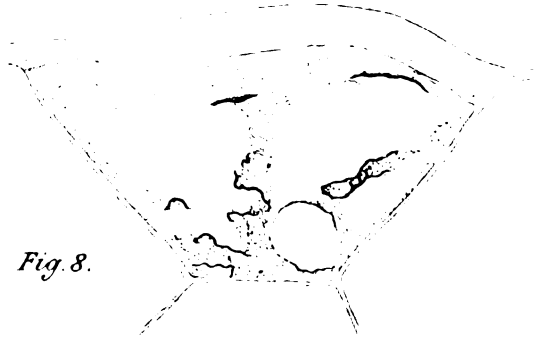

Fig. 11.

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GROOM.— ON DISCHIDIA RAFFLESIANA.

Fig. 5.

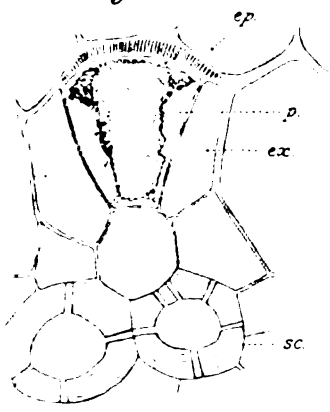


Fig. 7.

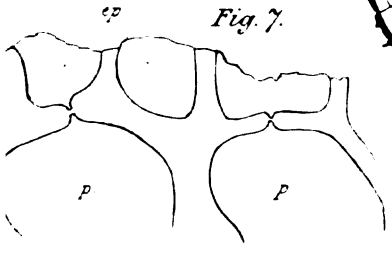


Fig. 9.

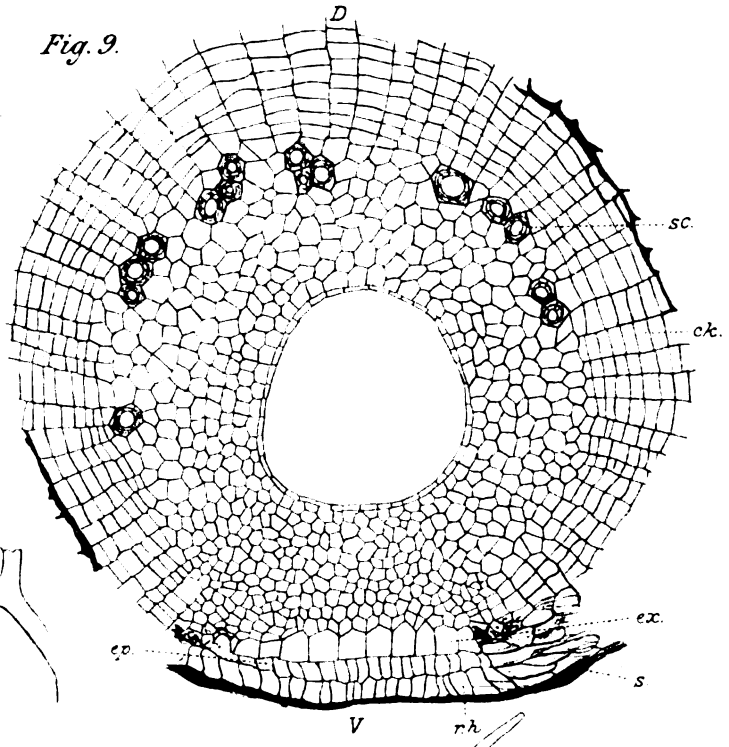
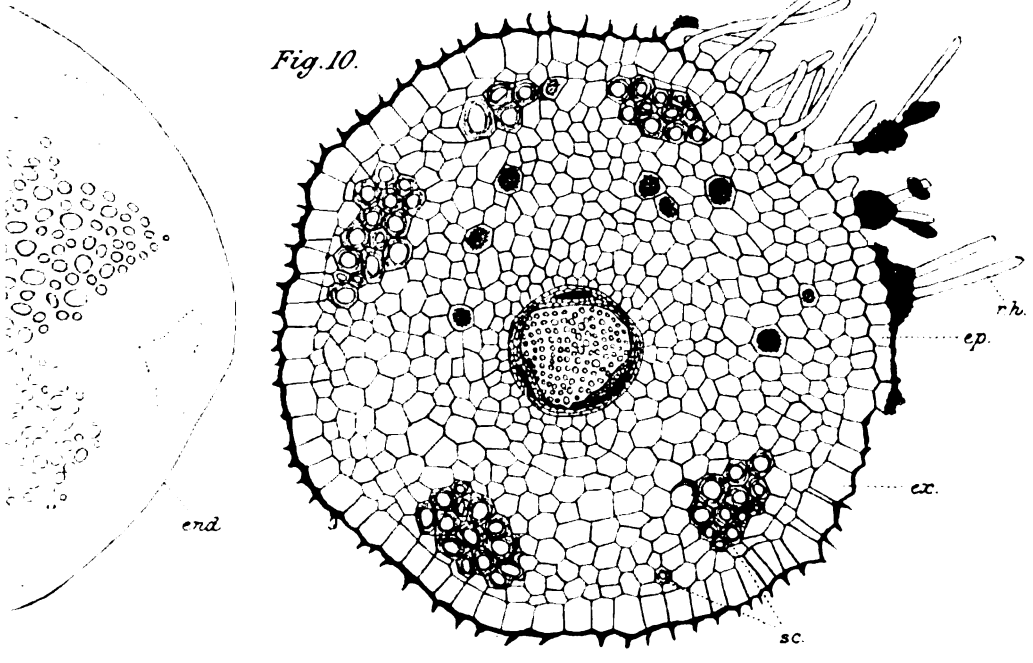


Fig. 10.



University Press, Oxford

On the Pitchers of Dischidia rafflesiana (Wall.).

BY

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AND

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With Plates XI and XII.

INTRODUCTION.

THE following extract from the Bulletin of the Royal Gardens, Kew, will sufficiently explain how our present investigation came to be undertaken[1]:

'*Dischidia rafflesiana.* After many unsuccessful attempts to introduce living examples of this interesting plant, Kew has at last succeeded, thanks mainly to the generosity of Dr. Treub, the distinguished Director of the Botanic Gardens, Java, who sent a plant of it in a Wardian Case, two years ago. This plant is now established and growing freely, producing numerous large pitcher-like leaves, as well as the small normal *Hoya*-like foliage.' The presence at Kew of living specimens producing pitchers afforded an excellent opportunity for re-investigating these curious organs, as to

[1] Bull. of Miscellaneous Information, 1892, p. 284.

which considerable difference of opinion still prevails among botanists.

Dischidia rafflesiana is a twining epiphyte, growing on trees, and especially, according to Treub, on those with thin foliage. Its germination does not appear to have been observed, but so far as mature specimens are concerned, it is purely epiphytic, and not attached in any way to the soil. The twining branches are long and slender, bearing decussate leaves of two kinds, namely small, very caducous scale-leaves, and the normal foliage-leaves, which are fleshy, orbicular in form, and may reach about an inch in length. They, like the scale-leaves, fall very readily. The plant is attached to the tree on which it grows, by numerous adventitious roots, which at first arise close to the insertion of the leaves. Later on the whole adherent surface of the stem may produce adventitious roots. The internodes of the twining stem are of great length. The pitchers are borne on short and thick lateral shoots. Sometimes two pitchers are produced at the first node of such a shoot, sometimes a pitcher arises on one side, and an ordinary leaf on the other. The pitcher-bearing branch usually grows very slowly, and its internodes remain for a time extremely short, several successive pairs of pitchers often being formed at its nodes. Sooner or later however the branch ceases to produce pitchers, and then grows out into an elongated twining shoot of the character usual in the plant. A full description of the habit and external morphology will be found in Treub's paper[1].

The pitchers themselves are of great size compared with the normal leaves, usually attaining a length, when mature, of about four inches. The pitcher, which is attached to the stem by a short stout petiole, forms an elongated, somewhat flattened sac, with a narrow opening at the end adjacent to the petiole. The edges of the pitcher are turned in at the mouth, the fold thus formed considerably narrowing the entrance. Fig. 1 gives some idea of a young pitcher as seen

[1] Sur les urnes du *Dischidia rafflesiana* (Wall.). Annales du Jardin Bot. de Buitenzorg, vol. iii. 1883, p. 13.

in longitudinal section. Excellent figures showing the habit will be found in the works of previous writers on the subject, especially in those of Wallich, Treub, and Beccari[1]. One or more adventitious roots arise at the base of the pitcher, and grow into its cavity, branching abundantly within it. Many of the pitchers are pendulous, others are horizontal, and some stand erect, with the opening turned downwards.

We do not propose to discuss the older literature, a sufficient account of which will be found in Treub's memoir. The principal papers, in addition to Wallich's original description of the species, are cited below[2].

As regards the morphology, Treub showed, by a study of the development, that the pitcher is a modified leaf, in which the inner surface corresponds to the lower surface of the normal foliage-leaf. He shows how the apical growth of the young pitcher is early arrested, and succeeded by an extremely vigorous intercalary growth of the middle portion of the organ, the growth being greater towards the morphologically upper surface, which thus becomes convex, while the lower surface becomes more and more concave. All stages of the process are figured in his paper. In the mature pitcher, therefore, the morphological apex is situated in the median line of the infolded margin at the mouth of the pitcher, at a point exactly opposite the point of attachment of the petiole to the lamina (see our Fig. 1).

On the question of the physiology of the pitchers, Treub rejects alike the hypothesis of Beccari, who regards the

[1] Wallich, Plantae asiaticae rariores, 1831, vol. ii. Tab. 142. This is a magnificent plate, and gives a most impressive idea of the habit of a fine specimen; Treub, loc. cit.; Beccari, Malesia, vol. ii. Piante Ospitatrici, 1886. Various species of *Dischidia* are figured.

[2] Robert Brown, Miscellaneous Works, vol. ii. p. 357 (published originally in 1832). This paper is on *Cephalotus*, and only contains a short reference to *Dischidia*; Griffith, Ascidia and stomata of *Dischidia rafflesiana*, Trans. Linn. Soc. 1851, vol. xx. Also in Notulae, vol. iv. 1854, where several species of *Dischidia* are described; Morren, Morphologie des Ascidies, Ann. Sci. Nat. Bot. Sér. II, t. xi. 1839; Beccari, Malesia, vol. i. His more recent and important work in vol. ii. has already been cited. Delpino, Nuovo Giorn. Bot. Italiano, vol. iii. 1871. A more recent paper is cited below.

pitchers as galls which have become hereditary and which may be useful to the plant as sheltering defensive insects, and that of Delpino who attributes to the pitchers a carnivorous function. Treub's own view is that the pitchers serve to collect rain-water, and to economize the watery vapour given off in transpiration; the detritus which he sometimes found in the pitchers may also serve as a supply of food. The roots he regards as serving for the absorption of the water, and possibly other substances contained in the pitchers. The walls of the pitchers are coated with wax, and are probably quite incapable of absorption.

Since Treub's paper appeared, the most important work published on *Dischidia* has been that of Beccari[1]. He maintains his former views, and after summing up the various possible functions which may be attributed to the pitchers, comes to the conclusion that they serve mainly as ant-shelters. He also found numbers of Acari on a very young pitcher, and suggests that they may regularly visit the pitchers at a very early stage, deposit eggs there, and thus excite the characteristic development of the organ. Beccari's view may be referred to shortly as the myrmecophilous theory. He considers the glandular processes found on young leaves and pitchers as 'food-bodies' comparable with those of *Acacia cornigera*, and suggests that they help to attract ants which effect fertilization.

Delpino has published a recent paper in which he further supports the view that the plant is carnivorous, the pitchers serving to catch insects, which are drowned, and then help to nourish the plant. He appears to rely on the form of the pitcher, which according to him is well-adapted to catch insects, but to this idea there is the evident objection, already made by Treub, that the roots afford a most convenient mode of escape to any insects which might happen to find their way into the pitchers[2].

[1] loc. cit., Malesia, vol. ii.

[2] Delpino, Malpighia, vol. iv. 1890, pp. 13–17.

Dischidia rafflesiana is also referred to in recent works by Göbel[1] and Schimper[2].

Our own object has been to make a careful anatomical study of the pitchers, and of other organs of the plant, so far as seemed necessary for purposes of comparison. Although the morphology of the pitchers appeared well-established, a study of the histological structure at different stages was likely to afford additional evidence of value. We also anticipated that a detailed investigation of structure would throw considerable light on the functions to which the organs are adapted, and in this anticipation we have not been disappointed. As usually happens, other points of interest arose during the investigation.

Structure of the Stem.

Before proceeding to compare in detail the anatomical structure of a pitcher with that of a normal foliage-leaf, it will be desirable to describe, very shortly, the anatomy of the stem, in order that the relations of the appendicular organs to the axis may be understood.

The stem shows the structure usual in Asclepiads. The vascular cylinder, in the young condition especially, is sharply marked off from the cortex, the innermost layer of which forms a starch-sheath or endodermis. The vascular bundles are only distinct from each other while quite young. The inter-fascicular tissue soon becomes completely converted into xylem, which forms a continuous ring surrounded by a circle of very numerous groups of normal phloëm. As in all the Asclepiadeae, internal phloëm is also present[3], and is in fact considerably more abundant than that in the normal position. The groups of internal phloëm show no constant relation to the protoxylem-elements. They are separated from the inner edge of the wood by at least two layers of parenchyma.

[1] Pflanzenbiologische Schilderungen, i. pp. 230–236.

[2] Die epiphytische Vegetation Amerikas, p. 22.

[3] Cf. Treiber, Üb. d. anat. Bau des Stammes der Asclepiadeen, Bot. Centralblatt, Bd. 48, 1891.

Laticiferous tubes, of the branched, inarticulated type usual in the Asclepiadeae, traverse both cortex and pith, and sometimes enter the phloëm-strands. The pericycle is several layers of cells in thickness; in its outer region there are numerous groups of sclerenchymatous fibres. The starch in the endodermal cells evidently serves to provide the material for the thickening of the cell-walls of the sclerenchyma; it is very abundant before this process begins, and gradually diminishes as the thickening layers are deposited.

That part of a lateral branch which bears the pitchers is considerably stouter than the stem generally. Where a pitcher is borne on one side of the branch, and an ordinary leaf on the other, the xylem is at least twice as thick on the side towards the pitcher, and on this side the amount of sclerenchyma is also enormously greater.

The number of vascular bundles entering a rudimentary scale-leaf is usually four. The normal foliage-leaves and the pitchers receive about six or seven bundles each. The outgoing bundles are accompanied by the internal groups of phloëm corresponding to them, and are imbedded in conjunctive parenchyma continuous with that of the stem-cylinder; the whole vascular system entering the petiole thus forms a *meristele*, in the sense of Van Tieghem [1].

Comparative Anatomy of the Foliage-leaf and the Pitcher.

The orientation of the vascular bundles in pitcher and leaf completely confirms the morphological conclusions arrived at by Treub from his study of the development.

The main bundles in both organs are bicollateral; in the smaller bundles the internal or superior phloëm becomes much reduced; in the finest branches it disappears altogether. In the case of all except the finest bundles the orientation was therefore determined by the position of the protoxylem-elements. In the leaf these are of course always di ected

[1] Journal de Botanique, t. v. 1891, p. 284.

towards the superior surface; in the pitcher they are as constantly turned towards the outer surface. The anatomy thus indicates plainly that the inner surface of the pitcher corresponds to the lower surface of the leaf.

As regards the distribution of the bundles in the lamina there is also a complete agreement between the two organs. The ramifications, which are not very abundant, traverse the middle layers of the mesophyll, without ever approaching closely to either surface. In the leaf however the minor branches are rather nearer to the upper than to the under surface, and in like manner the corresponding bundles in the pitcher lie nearer to the outer surface than to the inner. In both organs blind endings of the finer branch-bundles occur, as is usual in Dicotyledons. These also lie in the middle of the mesophyll.

As regards the mesophyll itself there is a considerable difference between the pitcher and the leaf. The lamina of the leaf is about twice as thick as that of the pitcher. The texture of the latter is much harder than that of the former. This is due in part to the sclerenchyma, to be described immediately, but in part also to differences in the parenchyma of the mesophyll.

In the foliage-leaf the tissues towards the upper and lower surfaces are alike, except that towards the upper surface the cells contain more chlorophyll-granules. Towards the middle of the mesophyll the cells are large, and somewhat elongated vertically to the surfaces: the cells here contain very few chlorophyll-granules. The mesophyll of the leaf, as a whole, is suggestive of an aqueous tissue, in which water may be stored.

In the pitcher the cells are altogether smaller than in the leaf, and have thicker walls. Towards the outer surface they are closely packed and rich in chlorophyll. Near the inner surface the cells are irregularly branched with numerous triangular spaces between them. Figs. 2 and 3 represent, in sections parallel to the surface, the outer and inner layers of the mesophyll.

The mesophyll bordering on the inner surface of the pitcher is the only tissue in the plant which appears to be especially adapted to transpiration. This fact is correlated with the distribution of the stomata, as we shall see below.

The layers next the inner surface of the pitcher contain a purple pigment, which was very slightly developed in the comparatively young pitchers formed at Kew, but was very abundant in pitchers of the same plant which had been already formed in Java and were about two years old. It was also very conspicuous in full-grown pitchers from other sources. No such pigment is present in the foliage-leaves.

Bands of thick-walled, but unlignified sclerenchymatous fibres accompany the bundles of both pitcher and leaf, chiefly on the inferior side. They form a sheath, crescent-shaped as seen in transverse section, enclosing the normal phloëm. Some of these fibres straggle off, as it were, from the bundles, sometimes singly, sometimes two or three together, and traverse the mesophyll in all directions. The fibres of the mesophyll therefore, are not isolated idioblasts, but are always continuous with the sclerenchyma of the bundle-system. The sclerenchyma is better developed in the pitcher than in the leaf.

Laticiferous cells extend into the mesophyll of both leaf and pitcher. They are much more abundant in the latter than in the former. In the leaf indeed they are so scanty that the latex is scarcely noticeable when the leaf is cut, while in the pitchers, and especially in the older ones, it spurts out as soon as the knife pierces the tissues. The main laticiferous tubes are found in the mesophyll above and below the midrib, and their principal branches traverse the middle layers of the mesophyll throughout the lamina. Numerous minor branches are sent out towards both surfaces, but they do not seem to accompany the finer bundles. In the pitcher the outer surface is much better supplied with laticiferous tissue than the inner. Some attempt was made to estimate the relative frequency of the tubes in the two regions. Branches were met with about twice as often within eight cells of the

outer, as within the same distance of the inner surface. On the outer surface the laticiferous tubes often approach very near to the stomata; on the inner surface, though the stomata are more numerous, this rarely occurs. The distribution of the laticiferous tissue may *possibly* indicate a relation to the assimilating system, but other explanations are not excluded.

We will next consider the *epidermis* of the two organs. In the leaf the cuticle is of the same thickness on both surfaces, in the pitcher; it is, as one would expect, thicker on the outer than on the inner surface. As regards the number of stomata, the outer and inner surfaces of the pitcher present a marked difference, which is entirely wanting if we compare the corresponding sides of the leaf.

In the case of the leaf, the number of stomata on the upper and under surface was found to be the same, being as nearly as possible 26 per sq. mm. for each, taking the mean of 43 numerations.

In the pitchers of the specimen from Java the average number on the outer surface was 16·5 per sq. mm. (mean of 38 countings) and on the inner surface, 33·5 (mean of 39 countings).

In some pitchers of *D. rafflesiana* sent to Kew from the Shan states of Burmah the difference was still more marked, the numbers being: outer surface 21·7 per sq. mm.; inner surface 57·2 per sq. mm. (mean of 15 countings in each case).

We see then that the inner surface of the pitcher is characterized by its structure as the *transpiring* surface *par excellence* of the plant, this being indicated both by the presence of spongy parenchyma in this region only, and by the relatively large number of its stomata.

On the outer surface of the older pitchers we occasionally observed patches of periderm, developed under the stomata. In these cases the stomata were sometimes replaced by lenticels.

In some instances a formation of callus on the inner surface of the pitcher was observed, as described by Treub. This was no doubt the result of injury.

The structure of the *petiole* of pitcher and leaf is essentially the same. The meristele of the pitcher is the larger and its xylem very much more abundant, as we should expect from the much greater size of the organ to be supplied. In the petiole of the leaf the meristele is slightly concave towards the upper surface; in that of the pitcher the curvature is much more marked.

It is interesting to find that in the pitcher-petiole there is an active cambium between the xylem and the normal phloëm, by which new elements are continually added to both tissues, the older phloëm-elements consequently becoming obliterated. There is also a cambial increase of the superior or internal phloëm, with resulting obliteration. Nothing of this kind occurs in the petiole of the ordinary leaf. This difference is evidently correlated with the long duration of life of the pitchers as contrasted with the transitory existence of the very caducous leaves.

The Purple Coloration of the Inner Surface of the Pitcher.

This was especially studied in the specimens from Burmah. The pigment was very well developed, giving the inside of the pitcher a fine deep purple colour, contrasting strikingly with the pale green of the outer surface. The pigment, which is in solution in the cell-sap, is limited to a thin layer of the mesophyll next the inner surface.

The spectrum of the light transmitted through the pigment was observed by means of a Zeiss micro-spectroscope.

Observations were first made on the whole thickness of the pitcher-wall, in order to determine the character of the light which actually reaches the interior of the pitcher through its wall, in nature. The light which penetrated the thick mass of tissue was however too faint for spectroscopic analysis.

Next, the thin purple inner layer was cut away from the thicker outer green layer, and each examined by itself. The intensity of the light passing through the green and red layers appeared to be about equal, notwithstanding their great differ-

ence in thickness. The green layer showed the ordinary chlorophyll absorption-spectrum; the purple layer gave a spectrum which differed most obviously from that of chlorophyll in the absence of the characteristic absorption-band in the red, from B to beyond C.

More accurate observations were made on an alcoholic extract of the purple pigment. The absorption-spectrum, in bright sunlight, was visible from ·71 to ·435 on the scale used, which was divided according to wave-lengths, that is, from midway between *a* and B, nearly to G.

From ·71 to ·589 (the D line) there was little or no absorption, the lines B, C and D being very clear. From ·589 to ·516 (about *b*) there was considerable absorption, the lines E and *b* being faint but distinct. From ·516 to ·435 (that is, from *b* nearly to G) the absorption was much greater, and no Fraunhofer's lines were visible. The whole violet end of the spectrum was completely absorbed.

We see then that the green and red layers between them cut off almost all light from reaching the interior of the pitcher; in particular, the blue and violet rays are most completely absorbed.

It is evident that the pitcher thus forms a dark chamber, into which the negatively heliotropic root is likely to be attracted, the darkness being most complete as regards those rays by which the direction of growth is influenced. The darkness of the pitcher may serve, not only to attract the adventitious root in the first instance, but also to ensure that its branches remain within the recesses of the organ. We do not overlook the fact that the presence of moisture and food must also be of influence in determining the direction of growth of the pitcher-root, and its branches.

Development of the Pitcher.

The development of the pitcher was studied in some detail. The youngest undoubted pitcher available was about $\frac{1}{8}$ in. (3 mm.) in length. The lamina was curved like a shell as in Treub's Fig. 1*a*, Pl. IV, the apex pointing downwards, and

almost at right angles to the axis of the organ; the part just behind the apex was already slightly hooded. The pitcher was cut, by means of the Cambridge rocking-microtome, into a series of transverse sections. The whole course of the vascular bundles in the young pitcher was thus completely disclosed, but it would be superfluous to enter into any full description, as the structure is in all respects that of a typical Dicotyledonous leaf of the 'isobilateral' type. There was no strictly apical growth at this stage; the morphological apex was surmounted by a gland, to which we shall return. The growth was most active in the hooded part just behind the apex, and here the tissues showed the least differentiation.

Another pitcher, which with its petiole was about $\frac{1}{2}$ in. (1 cm.) long, was microtomed in the longitudinal direction, parallel to the plane of symmetry. At this stage there is no definite localization of growth. The whole curved part of the organ seems to be actively developing, the mesophyll-cells showing indications of recent transverse divisions. Only at the base and apex growth appears to have ceased. At this stage the pitcher has attained its definitive form. Up to this time the histological structure of the pitcher is just that of a young foliage-leaf; the differentiation of the mesophyll, described above, only takes place at a later stage.

These serial sections, showing the whole structure of the pitcher at the stages in question, were prepared with the special object of determining whether any glandular organs are present, which might have been overlooked by previous observers. The careful examination of mature pitchers, in hand-sections, indicated nothing of the kind, but the only way of proving a negative was to examine the whole organ throughout, and this is just what the series of microtome-sections enabled us to do. The pitchers examined in this way were of necessity young ones. In the pitcher $\frac{3}{8}$ in. long however all the definitive tissues were present; the mesophyll had attained its full thickness, and many of the stomata were almost fully developed. No signs whatever of any developing glands were present on any part of the pitcher.

The pitchers, in common with the ordinary leaves, possess numerous glandular bodies, but these are without exception transitory, and belong only to the developing organ, ceasing to be functional long before the pitcher is mature. To guard against the possibility of any misconception as to the significance of these glands, we have thought it well to describe and figure all the forms in detail.

The Glandular Organs.

The secretory organs of *Dischidia rafflesiana* are of two kinds—hairs and emergences. The former may very soon be disposed of. Hemispherical, unicellular glandular hairs, secreting mucilage beneath the cuticle, are frequent on both surfaces of pitchers and leaves alike; they disappear very early, and in fact only a few were left even on a pitcher $\frac{1}{8}$ in. in length, while on that $\frac{3}{8}$ in. long it was very rare to find one which had not collapsed. Woolly protective hairs are also present on the outside of the young foliar organs.

The secretory emergences occur in three different positions; we find *petiolar*, *laminar*, and *apical* glands. The two former, but not the latter, have been described by Treub and earlier writers.

The petiolar glands occur at the base of the petiole of all three kinds of foliar organs, scale-leaves, foliage-leaves, and pitchers. The glands are two in number, seated one on each side of the petiole, at its base, so that, as Treub says, they seem to arise half from the petiole and half from the stem. The form of the gland is usually conical; its structure is extremely simple. The gland consists of a uniform thin-walled parenchyma, which is covered by a layer of columnar epithelium, the cells of which are rich in protoplasm and have each a fairly large nucleus. The secretion, both of these glands and of those in other positions, is mucilage, with a trace of resin. All the organs of the bud are imbedded in this mucilage. No trace of vascular tissue was ever found in a petiolar gland. The structure of these glands is shown in Figs. 4, 5, 6, and 7.

The petiolar glands develop early, and soon cease to be functional. They are for a time almost equal in size to the young leaves themselves. They were present and in full activity on the very young scale-leaves of the bud figured, in transverse section, in Fig. 5, *pg*. They were also present and functional on the foliar organs of the bud from a pitcher-bearing stem, shown, in longitudinal section, in Fig. 4, *pg*. These organs may be either young pitchers or foliage-leaves; the stage is too early for us to be certain, but from the position of the bud, on a branch which has already produced a pitcher, the presumption is rather in favour of these organs being themselves young pitchers[1].

On the pitcher $\frac{1}{8}$ in. long, already described, the petiolar glands were still present, but a phellogen, forming cork, had already arisen below the secreting epithelium, so that the gland was at this stage ceasing to be functional.

On the pitcher $\frac{2}{3}$ in. long, the petiolar glands, though present, were completely out of function; the walls of the epithelium were much thickened; a well-marked hypodermal layer of flattened, thick-walled cells had arisen from the divisions of the phellogen. Treatment with strong sulphuric acid showed that all the walls of the epithelial layer, and all but the inner walls of the hypodermal layer, were strongly cuticularized (see Fig. 7). In the older leaves and pitchers these glands are entirely absent, a scar marking their former position.

The *laminar* glands are somewhat later in development than those on the petiole. In the apical bud shown in Fig. 5 they were not yet developed. At the first node, immediately below the actual bud, they were present in an early stage of development. At the next node below this they were already functional, though not yet mature. Their number is variable; in the scale-leaves we found two or three, on the foliage-leaves and pitchers four or five. The size of the mature glands is also very variable, and so is their shape, which is generally rather conical, often narrowed at the base, where the epithelial layer ceases (see Fig. 8). These glands are seated on a cushion

[1] Cf. Treub, l. c. p. 19.

or shelf, rising from the upper side of the lamina, just above the petiole (see Fig. 1). On a young foliage-leaf ½ in. long they had already disappeared, only the cushion and scars marking where they had been seated.

They were present and functional on the various young pitchers examined, up to a length of 1½ inches.

In pitchers developed at Kew, and not yet mature, as shown by the rudimentary roots, absence of pigment, and other signs of youth, the glands had ceased to be functional, the epithelial cells being themselves cuticularized, and also severed from the inner tissue by a layer of cork.

Laminar glands (*lg*) are shown in Fig. 4, in the longitudinal section of the apex of a pitcher-bearing shoot, in Fig. 6, in a transverse section through a young node with scale-leaves, and in Fig. 8 from a longitudinal section of a pitcher ⅜ in. in length.

The relation both of the vascular bundles and of the laticiferous tubes to the laminar glands was traced. It is usual for the vascular bundles entering the foliar organs to approach rather nearly to the cushion in which the glands are seated; in many cases a branch runs off to the base of a gland, and is often continued up into the gland itself, but usually only in the form of a strand of elongated cells, not otherwise differentiated. In one case only was a spiral vessel found entering a gland (see Fig. 8).

Branches from the laticiferous cells often approach the glands, and sometimes enter them. This also, however, is evidently an inconstant phenomenon.

The third type of gland has not, so far as we know, been observed before. This is the *apical* gland, which is seated directly on the morphological apex of leaf and pitcher, just beyond the termination of the midrib. It is present on all the three kinds of leaves. The apical glands are developed extremely early; thus they are present, and to all appearance functional, on the scale-leaves (l_2) shown in transverse section in Fig. 5. Of course the section figured does not pass through these glands.

In the case of the foliage-leaves the period of development was not determined; a mature leaf bore on its apex the scar where the gland had been seated.

On the foliar organs (probably young pitchers) shown in Fig. 4, the apical gland is fully developed and functional (Fig. 4*a*); the glands are also present on the still younger leaves, decussating with those shown in median section. On the pitcher $\frac{1}{8}$ in. long the apical gland is present and functional (see Fig. 9). The midrib approaches very closely to the gland, closer than is shown in the figure, which is from a rather oblique section. On the pitcher $\frac{3}{8}$ in. in length the apical gland was quite withered. It is afterwards completely cut off by cork.

The organs enumerated are the only glandular structures on the vegetative parts of *Dischidia rafflesiana*. It is quite evident that they have nothing to do with any function of the mature pitcher, first, because they occur indiscriminately on ordinary leaves and on pitchers, and secondly, because they become functionless and are cut off by periderm, long before the pitcher is mature. They are evidently comparable with the colleters and other glandular organs so common on buds. Their immediate function is the secretion of mucilage, which is no doubt useful to the plant by preventing the desiccation of the delicate embryonic organs, and possibly also by rendering them distasteful to certain animal enemies[1].

These transitory glands can certainly have no relation either to a carnivorous or myrmecophilous habit. Our conclusions are in complete agreement with those of Treub[2].

The Roots.

The roots of the mature plant are entirely adventitious. The ordinary adventitious roots, already mentioned, serve as organs of attachment, by which the epiphyte is firmly fixed to the tree or stump round which it twines. They are at the same time organs of nutrition. They often form tangled

[1] Cf. Stahl, Pflanzen und Schnecken.

[2] l. c. p. 15.

clumps, in which particles of vegetable detritus and other substances become entangled. The root-hairs lay hold, in the usual way, of the particles of the soil thus accumulated.

Of greater interest, however, are those adventitious roots which enter the pitchers. The petiole of a pitcher usually bears two roots, one of which grows out on the lower convex side of the petiole, while the other appears on the opposite side, or laterally. The former is the one which usually enters the pitcher. The other generally remains outside, and behaves as an ordinary free root. In some cases, however, more than one root may enter a pitcher, and no doubt more than two may sometimes arise on its petiole.

The question whether the pitcher-roots have the same insertion as the ordinary roots, appeared to us to be of interest, for this involved the further question whether the two kinds of root are distinct at their first orgin, or become differentiated later. According to Treub there is a difference of insertion, the ordinary roots arising from the stem, and the pitcher-roots from the petiole[1]. So far as the external attachment is concerned, this is the case, but the question of the actual place of origin of the root remained. We investigated the point carefully, by means of serial sections through pitcher-bearing nodes. We found that there is no constant difference in the insertion. The tissues of all the adventitious roots examined are inserted on the cylinder of the stem very near the point where the leaf-trace bundles begin to bend out. Usually the insertion is rather below this point; occasionally it is slightly above it, but such differences are inconstant. The pitcher-root grows for some distance through the parenchyma at the base of the petiole, before becoming free. It attains maturity much more slowly than an ordinary adventitious root, a fact which no doubt finds its explanation in the extremely slow maturation of the pitcher itself, as compared with that of a foliage-leaf.

The roots which enter the pitchers branch repeatedly. It generally happens that a considerable amount of detritus of

[1] l. c. pp. 15 and 18.

various kinds finds its way into the pitcher, possibly washed there by rain from the bark of the tree on which the plant is growing. This detritus forms a rich natural soil at the bottom, or on the sides of the pitcher, and into this soil the rootlets direct their growth. Where a thin layer of humus coats the inside of the pitcher, the rootlets apply themselves closely to this surface, becoming flattened, and often acquiring a somewhat dorsiventral structure.

The pitcher-roots, as we should expect, do not simply absorb water, but are also able to make use of the supplies of food with which the soil in the pitcher provides them. The root-hairs attach themselves firmly to particles of humus, and in fact behave just as root-hairs in an ordinary soil would do (Fig. 11).

The anatomy of the pitcher-root presents some points of interest. The vascular cylinder of its main axis is pentarch or tetrarch. In the specimens from Java the wood of the mature root extends to the centre, so that there is no pith. The wood consists of spiral vessels (limited to the protoxylem), tracheides with bordered pits, and xylem-parenchyma. The phloëm is well-developed and normal, the pericycle is one or two layers in thickness. The cortex of the root is persistent, no internal periderm having been formed in any of the specimens observed. The cortex contains laticiferous cells, crystal-sacs with calcium oxalate in clustered crystals, and numerous sclerotic cells. The latter are usually limited to the outer cortical layers, where they form irregular groups, as shown in Fig. 10. These cells are often thickened almost to the obliteration of their lumen, with narrow pits. The short cells have square or slightly oblique walls. They do not form long continuous series in the longitudinal direction, and are sometimes quite isolated. They can scarcely contribute much to the mechanical strength of the root, and in fact no great strength is needed. They harden the external cortex, however, and may possibly serve a protective function.

Outside the layer containing the sclerotic cells is the exodermis, which in the mature parts of the root forms the

external surface, as the piliferous layer soon perishes. Later on again, an external periderm is formed, usually originating in the cells next below the exodermis. Subsequently peridermal arcs may be formed further towards the interior, by which groups of sclerotic cells are often excised. In all the cases observed, however, the greater part of the cortex was persistent.

The finer branches of the pitcher-root usually have a diarch or triarch cylinder. Where they are flattened against the sides of the pitcher their structure is somewhat dorsiventral, the cortex being decidedly thinner on the adherent than on the free side. The same peculiarity was also observed in the ordinary adventitious roots.

The exodermis is remarkable for its very characteristic passage-cells (see Figs. 12 and 13), which are arranged with great regularity in longitudinal rows. The form of the cells, as seen in radial section, appears conical, the convex base of the cone bordering on the piliferous layer, while the somewhat truncated apex abuts on a protuberance of the cortical cell below. The side-walls are in contact with the other exodermal cells.

The passage-cells have much denser protoplasm than the other exodermal cells. The cuticularization of the various cell-walls was tested, and it was found that all the walls of the exodermal cells are somewhat cuticularized, with the exception of the *inner* walls of the passage-cells. The *outer* walls of these cells are only partially cuticularized, the middle layers of the membrane giving cellulose reactions. The outer cell-walls of the piliferous layer, as well as those of the root-hairs themselves, are cuticularized[1]. It is evident that a thin layer of cuticle does not hinder absorption; as however the walls of the passage-cells are certainly less cuticularized than those of their neighbours, we may assume that they really have a special conducting function to perform.

The ordinary adventitious roots do not differ very strikingly, so far as we observed, from those in the pitchers. Their root-

[1] This is common in root-hairs. See Strasburger, Bot. Practicum, 2te Auflage, p. 280.

hairs, as mentioned above, attach themselves to any particles of humus which they meet with. The exodermis has fewer passage-cells in the same length, which may possibly indicate less active absorption. The rootlets, where they adhere to the substratum, are often much flattened, and sometimes show a dorsiventral structure. The sclerenchyma is mainly limited to the free side, while the external periderm first forms on the side towards the adherent surface. A particularly good example of the structure in question was found in an adventitious root of another species, *D. bengalensis*, Colebrooke[1], which we have therefore figured in transverse section (Fig. 14). On the whole the free adventitious roots have decidedly fewer sclerotic elements than the pitcher-roots.

Internal Phloëm in the Root.

The roots of the Java specimens showed no anatomical anomalies. It was therefore surprising to find that the roots in the pitchers of the same species sent to Kew from Burmah possessed well-developed medullary phloëm, a rare peculiarity in the root.

Three pitchers from Burmah were available for histological examination. In one the root was so shrivelled that its structure could not be investigated. Both the other two had internal phloëm.

All the roots in the Burmah pitchers were very well developed. They were abundantly branched, and their rootlets formed a tangled mass, pressed closely against the bottom and sides of the pitcher, among the detritus which it contained. When pulled out, the mass of rootlets retained the form of the pitcher, just as do the crowded roots removed from a flower-pot. The flattened rootlets were decidedly dorsiventral, the differences between the adherent and free sides being in all respects as marked as in the adventitious root of *D. bengalensis*, which we have figured (Fig. 14).

The main root in each pitcher was swollen here and there,

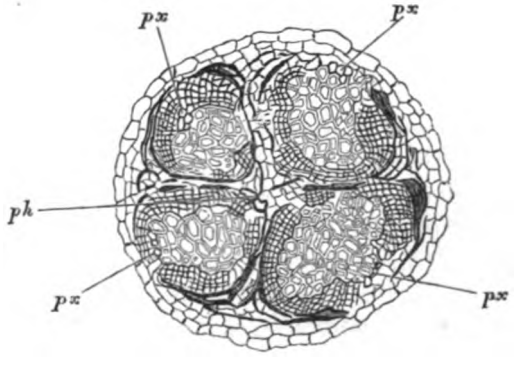
[1] Trans. Linn. Soc. vol. xii. 1819, p. 357, Pl. 15.

and did not begin to taper off regularly for about 4 cm. from its base. At this point the internal phloëm disappeared, becoming confluent with the normal external phloëm. It was also absent at the base of the root.

We will describe the anatomy in detail in one case, as both the roots showed similar variations in structure.

In this case the internal phloëm makes its appearance, if we trace the structure from base to apex, before the root becomes free from the parenchyma of the petiole. It is continuous with the normal phloëm, which is alone present at the actual base of the root, and through this, with the normal phloëm of the stem. The anomaly of the root is therefore a local one, as its medullary phloëm has no direct communication with that of the stem.

At 1 mm. from the apparent insertion of the root (i. e. from the point where it becomes free from the petiole) the structure has become very complicated. The tetrarch xylem here forms four distinct masses, each surrounded by a ring of phloëm, the external elements of each ring being at this stage obliterated (see Woodcut A).



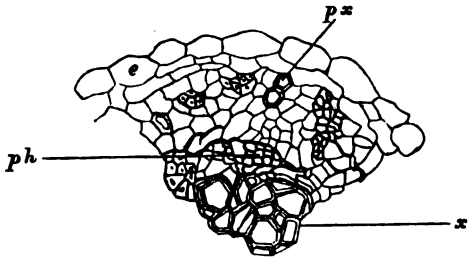
Woodcut A.

From transverse section of pitcher-root 1 mm. below apparent insertion: *px*, protoxylem; *ph*, phloëm. × 120.

In the middle of the root, between the four rings, is a little parenchymatous pith. This peculiar structure recalls the anomaly of the stem of *Acanthophyllum*, described by Dangeard [1]. Between each xylem-mass and the surrounding phloëm is a complete ring of cambium. The whole structure may be described as a 'pseudo-polystely.'

Between 6 and 7 mm. from the insertion the xylem-masses fuse laterally, and the internal phloëm comes to be completely enclosed by a ring of wood, while it itself encloses a few partly obliterated pith-cells at the centre. In this region the root is pentarch.

Further down, the arrangement becomes more complex again, the xylem-ring opening out, so that internal and



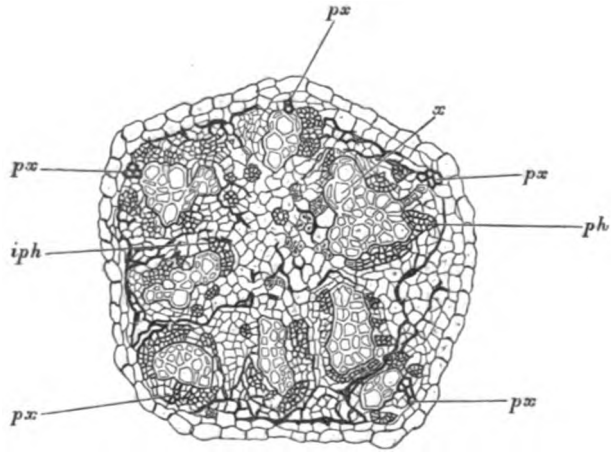
Woodcut B.

From transverse section of pitcher-root 20 mm. below apparent insertion: *px*, protoxylem; *x*, xylem; *ph*, phloëm. × 240.

external phloëm once more become continuous. An additional complication appears, owing to the fact that some of the protoxylem-groups are here separated from the other xylem, and the phloëm extends between the two (see Woodcut B, which is drawn from a section taken at 20 mm. from the base of the root). A few mm. lower down, the xylem is completely broken up into sixteen or more isolated groups, separated by phloëm. Woodcut C is drawn from a section at 33 mm. from the base, where the xylem has united again to form a smaller number of groups. The large total amount of phloëm in this part is striking.

[1] Le Botaniste, t. i. 1889.

At 38 mm. the structure is very much simplified; the xylem forms a continuous ring, except at one point where the medullary and the normal phloëm are continuous.



Woodcut C.

From transverse section of pitcher-root 33 mm. below apparent insertion: *px*, protoxylem; *x*, xylem; *ph*, phloëm; *iph*, internal phloëm. × 120.

Lastly, at 43 mm. from the base, the structure has again become quite normally tetrarch; there is a small pith here, but no internal phloëm. The branches of the root are altogether normal.

We cannot, of course, say whether the curious anomalies just described are characteristic of the Burmah form of the species. Possibly it is merely a matter of individual variation, for which analogies would not be wanting. In *Strychnos spinosa*, for example, one of us observed medullary phloëm in some roots and not in others of the same plant[1]. Jost and Schumann have found that medullary bundles may appear in the stem, as an individual peculiarity[2], in *Phaseolus* and *Carum*.

[1] Scott and Brebner, Anatomy and Histogeny of *Strychnos*, Annals of Botany, vol. iii. 1889.

[2] Jost, Bot. Zeitung, 1891, p. 485; Schumann, Bot. Centralblatt, Bd. 45, Heft 12, 1891.

From a physiological point of view, we can only say that those roots of *Dischidia rafflesiana* which have internal phloëm, are showing a tendency to become fleshy, as is indicated by their irregularly swollen form. These roots are evidently becoming adapted for the storage of proteids; whether the material for these proteids is derived from the humus in the pitchers, or from the tissues of the plant itself, it is impossible to say. The structure of the internal phloëm is perfectly normal, and is identical with that of the external phloëm in the same root.

Medullary phloëm has, we believe, so far only been recorded in the genera *Cucurbita*, *Vinca*, *Strychnos*, *Chironia*, and *Lythrum*[1]. It therefore seemed worth while to describe this new case of so exceptional an anatomical feature. Medullary phloëm in the root has always been found to be a very inconstant character. Strands of phloëm in the *wood* of roots are less uncommon and somewhat more constant when they occur.

Conclusions.

If we now endeavour to sum up the results of our work on the pitchers of *Dischidia rafflesiana*, we find that as regards the morphology, there is no difficulty. Treub's view is completely confirmed by the study of the anatomical structure. The pitcher is a modified leaf, formed by great intercalary growth of the whole region between petiole and apex, the morphologically upper surface growing more rapidly than the lower. The outer surface of the pitcher therefore represents the upper surface of the leaf, and the inner surface of the pitcher the lower surface of the leaf.

As regards the physiology, we can only be guided by the structure, as, under the artificial conditions of cultivation, experiment was out of the question.

There are no structural features whatever which lend the

[1] See Scott and Brebner, Internal Phloëm in Root and Stem of Dicotyledons, Ann. of Bot. vol. v. 1891. For *Lythrum* see Frémont, Journal de Bot. t. v. 1891, p. 448.

slightest support to the theories either of carnivorous or myrmecophilous habit. There is no provision either for attracting or detaining insects. The numerous glands which are present are all merely organs belonging to the bud, and have perished long before the pitchers begin to be functional. From Treub's observations at Buitenzorg, it would appear that the presence of insects in the pitchers is very inconstant.

Beccari's theory that the pitchers may be of the nature of galls receives no support from our observations, for the young pitchers developed quite normally at Kew, and no insects were found in them at any stage. It is beyond our province to discuss Beccari's gratuitous hypothesis of *hereditary* gall-formations.

Treub, as we have mentioned, takes the view that the pitchers serve to collect rain-water, and in a less degree to economize the water of transpiration. He also thinks that when detritus is washed by rain into the pitchers, it may be available as a supply of food.

We agree with the views of Treub. The upright pitchers, which we ourselves had an opportunity of observing in the specimen at Kew, can have no other function than to store up the water given off as vapour in transpiration. We have shown, on anatomical evidence, that the inner surface of the pitcher is the chief transpiring surface of the plant. The condensed water of transpiration is undoubtedly re-absorbed by the roots.

The obvious function of the pendent pitchers as catch-reservoirs of rain-water requires no further explanation.

We are disposed to attach considerable importance to the detritus in the pitchers. It was abundantly present in all the older pitchers which we observed, and the rootlets had directed their growth so as to make the greatest possible use of it. The behaviour of the root-hairs proves that the humus is actually used as a food-supply. We regard the function of soil-collectors or 'natural flower-pots' as by no means the least, perhaps rather the most important of those performed, at any rate by the pendent pitchers.

It is very desirable that observations should be made on some of the other pitcher-bearing species of *Dischidia*[1], which seem to be very imperfectly known.

The comparison of the pitchers in *D. rafflesiana* with the leaves of such species as *D. Collyris*, Wall. (= *Conchophyllum imbricatum*, Bl.), suggested by Beccari, Treub, and Goebel, is most instructive. There is a specimen of *D. Collyris* in the Kew Herbarium which shows a long series of the concave, shell-like leaves, each sheltering within its hollowed lower surface a mass of adventitious roots. The under-surface of these leaves has the purple coloration characteristic of the pitchers. We can scarcely doubt that from some such leaves as those of *D. Collyris*, the more highly-modified root-sheltering pitchers of *Dischidia rafflesiana* have been evolved.

[1] They are enumerated by Beccari, Malesia, vol. ii. p. 260.

EXPLANATION OF FIGURES IN PLATES XI AND XII.

Illustrating the paper on *Dischidia* by Dr. Scott and Miss Sargant.

D. rafflesiana.

Fig. 1. Longitudinal section of pitcher ¾ in. long, with median section of a laminar gland *lg*, and withered apical gland *ag*. × 6·6 (reduced from 20).

Fig. 2. Surface section just under epidermis of outer surface of Java pitcher. × 60 (reduced from 120).

Fig. 3. Surface section just under epidermis of inner surface of Java pitcher. × 60 (reduced from 120).

Fig. 4. Median longitudinal section of apex of pitcher-bearing shoot. *l*, *l'*, apices of smallest leaves; *l*, *l'*, next pair of leaves; *lg*, laminar gland; *pg*, petiolar gland. × 60.

Fig. 4 *a*. Apical gland on *l'*, from another section in the same series. × 120.

Fig. 5. Transverse section of apex of foliage stem. *l*, *l'*, youngest pair of scale leaves; l_1, l'_1, l_2, l'_2 successive pairs of decussate scale-leaves; *pg*, petiolar glands. × 120.

Fig. 6. Transverse section of glands on a leaf of the second node below the apex in the same stem as Fig. 5. *L*, leaf; *St*, stem; *pg*, petiolar glands; *lg*, laminar glands. × 120 (reduced from 240).

Fig. 7. Longitudinal section of petiolar gland from pitcher ¾ in. long. × 80 (reduced from 120).

Fig. 8. Longitudinal section of laminar gland from same pitcher. × 80 (reduced from 120).

Fig. 9. Oblique section of apical gland from pitcher ½ in. long. × 80 (reduced from 120).

Fig. 10. Transverse section from base of root in Java pitcher. × 120 (reduced from 240).

Fig. 11. Transverse section of part of rootlet in Java pitcher, showing root-hairs with particles of humus attached to them. × 180.

Fig. 12. Radial section from rootlet in Java pitcher, showing exodermis. *p*, passage-cell. × 120.

Fig. 13. Tangential section from rootlet in Java pitcher through exodermis. *p*, passage-cell. × 187·5 (reduced from 375).

D. bengalensis.

Fig. 14. Transverse section of adventitious root; root-hairs matted together on adherent surface, epidermis withered on free surface. × 80 (reduced from 120).

Fig. 1.

Fig. 2.

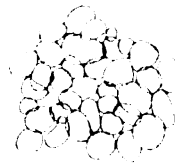


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Fig. 4.

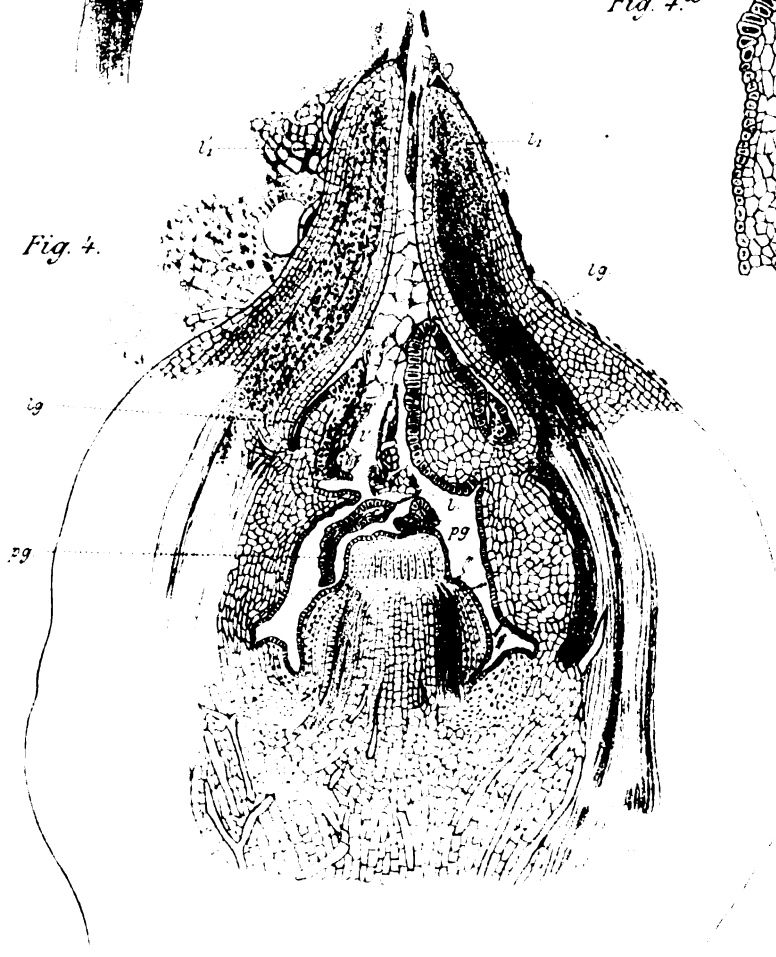

Fig. 4.a

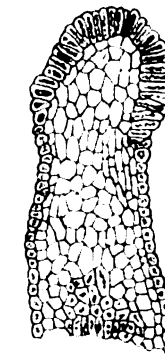

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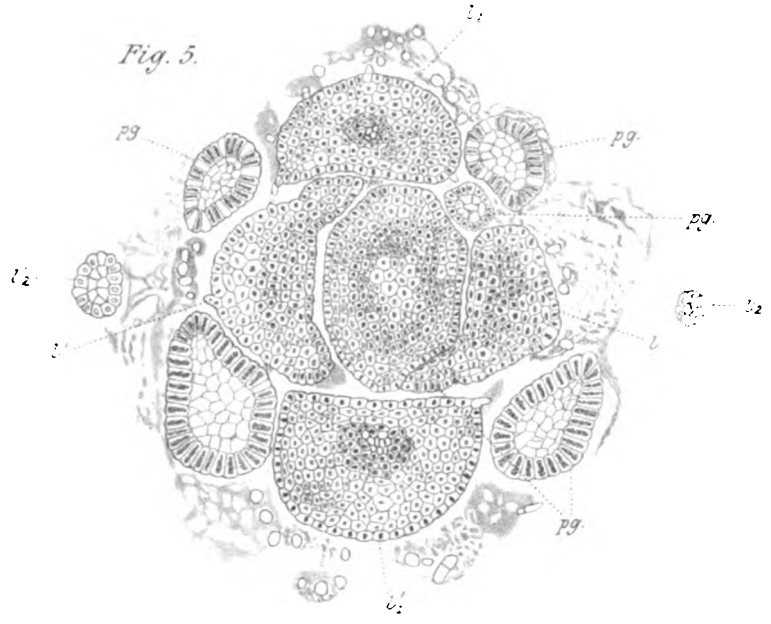
Fig. 5.



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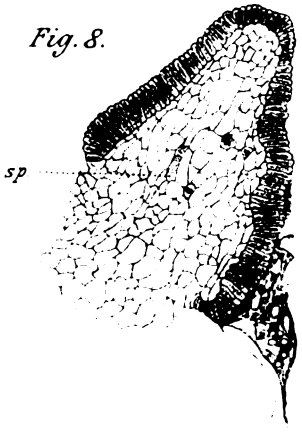

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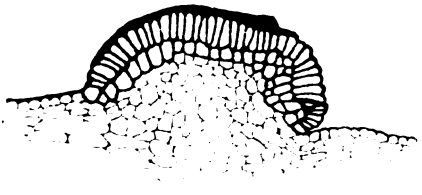

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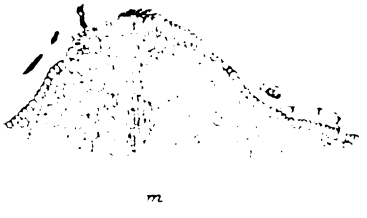

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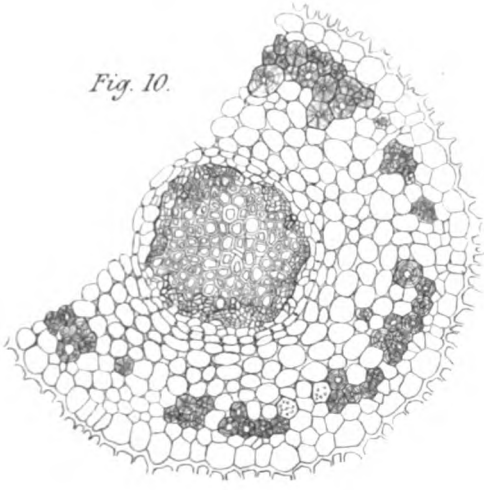
Fig. 10.



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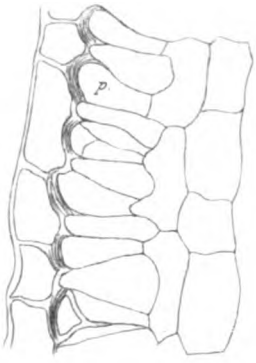

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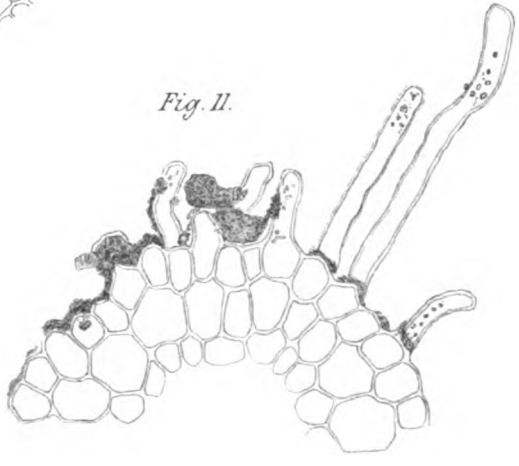


Fig. 13.

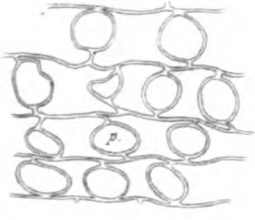
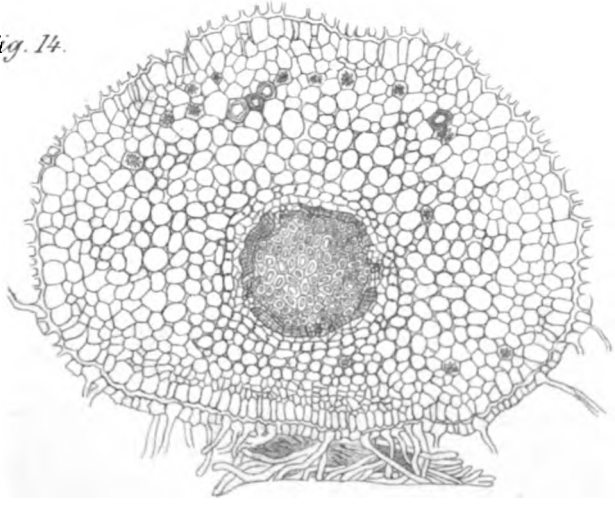


Fig. 14.



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NOTES.

A CONTRIBUTION TO THE CHEMISTRY AND PHYSIOLOGY OF FOLIAGE-LEAVES[1]: BY HORACE T. BROWN, F.R.S., AND G. H. MORRIS, PH. D.—The investigation of which we give an account in this paper is an attempt to throw some light upon the occurrence, relations, and physiological significance of the starch, diastase, and sugars contained in foliage-leaves.

It originated in an attempt to explain a certain technical operation in brewing which had hitherto been purely empirical in its application, and to this we may perhaps briefly refer at the outset, as there is a certain amount of chemical interest attached to it.

When the primary fermentation-process is completed and the beer has been racked into casks, it has been customary in some localities to add to the finished beer a small quantity of dry hops, the effect of which is marked in several ways. One very important effect is to hasten the secondary fermentation, and thus to produce a 'briskness' or 'freshness' in the beer, a fact which has been well recognized for generations by the practical brewer, but up to the present time has never been satisfactorily explained.

It is unnecessary to give any details of the investigation which led us to a true explanation of this effect of 'dry hopping,' as it is called, especially as we have recently treated of it at length elsewhere, but we may briefly state that we found it to be dependent upon the presence in the hop-strobiles of a small but appreciable amount of *diastase*, sufficient to slowly hydrolyze the non-crystallizable products of starch-transformation left in the beer, and to reduce them to a condition in which they can be seized upon and fermented by the yeast.

It now became a matter of interest to ascertain if this occurrence of diastase in the hop-strobile is an isolated case, or a special example of a widely distributed property of vegetable tissue.

[1] Abstract of a paper read before the Chemical Society, April 20, 1893.

An examination of all the published facts relating to the occurrence of diastase in plants revealed a curious number of conflicting statements, from which it was impossible to draw any conclusion without further investigation. The importance of such an investigation from a physiological point of view was manifest, and during its progress we found ourselves carried by degrees into all the vexed questions connected with the first formation of starch in the chloroplasts of the foliage-leaf, the mode of the dissolution of this starch and its translocation in the plant, and the nature of the metabolized products between the starch and the formation of new tissue.

Although our investigation is by no means as complete as we could wish, we think that the results so far obtained will not prove devoid of interest either to the chemist or the vegetable physiologist.

Of the previous work bearing upon the occurrence and formation of starch in the chlorophyll-bodies of green leaves undoubtedly the most important is that of Sachs, who, in a series of papers dating from 1862, clearly established the important fact that the appearance of so-called autochthonous starch in the chlorophyll-granule is induced by, and is dependent on, the action of light of sufficient intensity, and that the green colouring-matter of the chloroplast is as essential to the production of this starch as it is for the decomposition of carbon-dioxide. Sachs, in fact, for the first time clearly formulated the proposition that the production of starch in the chloroplast is directly connected with assimilation, and also showed that when plants are placed in the dark the starch disappears from their chloroplasts to re-appear again when light of a sufficient degree of intensity is once more allowed to fall on them.

The immense importance of these facts was duly appreciated by their discoverer, who was led by them to the further recognition of a periodic daily change in green leaves, by which the starch, formed in the chloroplasts during the hours of daylight, is wholly or partially re-dissolved and removed from the leaf during the night, to supply the constant demands of growth and respiration.

In his important paper of 1884[1], Sachs still further advanced our knowledge of the starch-building, and the subsequent metabolism of starch in the leaf. He devised an iodine-method, now well known, for roughly determining the relative amount of starch in the leaf,

[1] Ein Beitrag z. Kenntniss der Ernährungsthätigkeit der Blätter. Arbeiten des Bot. Instituts in Würzburg, 3. 1, 1884.

and also attempted to determine the total amount of starch formed or dissolved in a given time by observing the variation in weight of equal areas of the leaf-lamina cut from the two sides of a large leaf like that of the Sun-flower. As these variations in weight of the dry leaf were found to be correlative with the indications of the iodine-test, they were attributed to a loss or gain of *starch*. An examination of the method proves, however, that it cannot give us the actual amount of *starch* formed or lost within a given period, but that it really measures the *total assimilated products* which have entered or left the leaf. Sachs speaks of it throughout his 1884 paper as a gain or loss of *starch*, because he assumes (although he nowhere precisely states it in this form) that all the products of assimilation pass at one time of their history through the form of starch in the chloroplasts. We have, however, no proof that starch is an *essential link* in the chain of chemical products, beginning with the inorganic materials and ending with the form in which the products leave the leaf. That a large part of the first products of assimilation does pass through the starch-stage under ordinary conditions there can be no doubt, especially when assimilation is very active; but no sufficient experimental proof, or in fact proof of any kind, has been given of that constant and rapid flux of starch within the chlorophyll-corpuscle which must go on if this assumption of Sachs is correct. Our own work is strongly opposed to this assumption, and points to by far the larger amount of assimilated material never passing through the stage of starch at all.

The half-leaf gravimetric method of Sachs is nevertheless a very valuable one if we bear in mind its limitations, and it gives us a very good idea of the amount of material assimilated in a given time by a given area of leaf.

In the full paper[1] we have given the results of a considerable number of determinations by this process, and have also determined the limits of error of the method. Our results agree very closely with those of Sachs in showing that the leaves of the Sun-flower, for instance, can, under favourable conditions, assimilate at the rate of from 1·0 to 1·5 grams per square metre of leaf-area per hour. We have been able to show however that only a small portion of this assimilated material exists at any one time in the form of *starch*.

[1] Journal of the Chemical Society, vols. lxiii, and lxiv, No. 366, May 1893.

It is evident that if we are to get at any of the metabolic secrets of the leaf an accurate method of starch-determination is, at the outset, essential, since no reliance can be placed upon any of the iodine-tests except for very rough relative estimates.

We have found it possible to estimate the starch of leaves with great exactness by hydrolyzing it, with suitable precautions, with diastase, and then determining the products of hydrolysis in the usual way by means of the polarimeter and Fehling's solution. It will not be necessary to enter here into the details of this process, but we will give the results of two such determinations of starch in the same leaf, in order to show how concordant the results are.

In two analyses of the same sample of dried *Tropaeolum*-leaf we found:—

(1) 6·40 per cent. starch.
(2) 6·54 „ „ „

As a square metre of dry *Tropaeolum*-leaf weighs about 25 grams these amounts represent per square metre of leaf about 1·6 grams of starch.

Precautions have to be taken in drying the leaves for these determinations, for if the leaf is dried too slowly and at too low a temperature there is a considerable loss of starch during the process owing to certain functions of the living cells, especially the respiratory processes, continuing for some time. The leaves must be killed rapidly by a fairly high temperature, or better still, can be submitted for a short time to chloroform-vapour, after which they may be dried at from 30° to 40°.

In order to give an idea of the fluctuations in the amount of starch when the leaves are placed under various conditions, we give the results of a few determinations made on *Tropaeolum*-leaves:—

	Starch per cent.	Grams of Starch per sq. m.
(1) Leaves plucked at 5 p.m. on a sunny day	6·50	1·756
(2) Same leaves depleted in dark for 63 hours	2·05	0·554
Another similar experiment.		
(1) Leaves plucked in afternoon	5·42	1·464
(2) Leaves in dark for 60 hours	0·90	0·244

On determining the total amount of matter assimilated by a square

metre of *Tropaeolum*-leaves, under favourable conditions, we found it amounted to 7·2 grams per square metre in 8 hours, or about 0·9 gram per square metre per hour; so that we see the actual amount of starch present in the leaf at any one time represents only a small proportion of the 10 or 12 grams of material assimilated during a summer's day of sunshine.

This conclusion is even more strikingly shown in our experiments with the leaves of the Sun-flower, in which we determined the total amount of material assimilated in 12 hours of insolation, and also the actual increase in the amount of starch during the same period.

	Grams per sq. metre.
Starch at 5 a.m.	1·05
Starch at 5 p.m.	2·45
Increase in starch in 12 hours	1·40

Total products assimilated in 12 hours 12·0 grams.

During the time that one square metre of the leaf has assimilated 12 grams of material from the atmosphere, the *starch* has only increased by 1·4 grams. If, therefore, Sachs' idea is correct that all the assimilated products pass through the form of starch, the deposition and dissolution of that substance in the leaf-cells must take place at a most astonishing rate. We have however failed to find any proof of starch being a necessary link between the sugars of assimilation and the sugars of translocation; the probabilities all point to the starch being elaborated in the assimilating cells only when the supply of nutriment is in excess of local requirements, most of the assimilated products never passing through the stage of starch at all. Later on we shall have to say something about the nature of the substance from which the starch is elaborated by the chloroplasts.

We must now turn to the occurrence of *diastase* in the leaf, and consider the part it plays in the dissolution of the starch formed by the chloroplasts. A careful examination of this question was rendered all the more necessary since it has been strongly denied recently by Wortmann[1] that diastase plays any part in the dissolution and translocation of starch in leaves.

To prove his case Wortmann relies upon the following facts which he believes he has established.

[1] Bot. Zeitung, 1890, p. 582.

(1) The absence of diastase from leaves, or its occurrence in very small quantities only.

(2) That when diastase does occur it is of such a nature and so feeble in its action that, unlike the diastase of germinating grain, it cannot act upon the solid starch-granule.

(3) That when the vital functions of the protoplasm are temporarily arrested by depriving the cell of oxygen then no disappearance of starch takes place, which ought not to be the case if starch-dissolution is dependent upon enzyme-action.

As regards Wortmann's first statement, that diastase rarely occurs in the leaf, our experiments are directly opposed to this. So far from leaves containing little or no diastase, we have not found a single case where diastase was not present in sufficient quantity to transform far more starch than the leaf can ever contain at one time; in fact we shall see that diastase is frequently present to an extent sufficient to hydrolyze an amount of starch many times the total dry weight of the leaf itself.

Wortmann attempted to determine the presence or absence of diastase by an examination of the clear filtrate obtained from a few hours' maceration of the crushed leaves with water.

The tenacity with which the protoplasm holds the enzyme, and the disturbing influence due to tannin, which frequently occurs in the leaf, render this method quite useless. It is only by previously drying the leaves, and using the dried tissue in actual contact with the starch-solution, that the full diastatic activity of the leaf can be appreciated.

Although there seemed every probability that the products of hydrolysis of starch by leaf-diastase are identical with those yielded by the diastase of germinated grain, we were not justified in taking this for granted, especially as Vines[1] has recently stated that the sugar so produced is not dextro-rotatory. We have made very many experiments on this point, full details of which we have given in the paper. They prove, without room for doubt, that the products of starch-hydrolysis by leaf-diastase are identical with those brought about by malt-diastase. Moreover that the sugar so formed is really *maltose* we have proved by actually crystallizing it out, by the determination of its optical and reducing properties, and by the preparation of the maltoseazone with phenyl-hydrazin acetate.

[1] Annals of Botany, vol. v. p. 409, 1891.

We have also shown that leaf-diastase, like malt-diastase, cannot carry the hydrolysis of the starch beyond the point of maltose; it cannot form dextrose. Leaves however contain an enzyme capable of inverting cane-sugar.

We have made a great number of relative determinations of diastase in leaves, the results of which are given in the following table:—

1.	*Pisum sativum*	240·30
2.	*Phaseolus multiflorus*	110·49
3.	*Lathyrus odoratus*	100·37
4.	*Lathyrus pratensis*	34·79
5.	*Trifolium pratense*	89·66
6.	*Trifolium ochroleucum*	56·21
7.	*Vicia sativa.*	79·55
8.	*Vicia hirsuta*	53·23
9.	*Lotus corniculatus*	19·48
10.	*Lupinus* (? sp.)	3·51
11.	Grass with clover	27·92
12.	*Tropaeolum majus*	4·90
13.	„ „	4·96
14.	„ „	8·29
15.	„ „	9·64
16.	„ „	7·43
17.	„ „	4·25
18.	„ „	3·91
19.	„ „	3·68
20.	„ „	4·25
21.	*Helianthus annuus*	3·94
22.	*Helianthus tuberosus*	3·78
23.	*Funckia sinensis*	5·91
24.	*Allium Cepa*	3·76
25.	*Hemerocallis fulva*	2·07
26.	*Populus* (? sp.)	3·79
27.	Lilac	2·53
28.	*Cotyledon Umbilicus*	4·61
29.	*Humulus Lupulus.* Leaves	2·01
30.	„ „ Strobiles with seeds (1)	9·60
31.	„ „ „ „ (2)	7·95
32.	„ „ „ „ (3)	6·00
33.	„ „ Strobiles free from seeds	2·06

34. *Hymenophyllum demissum* . . . . . 4·20
35. *Hydrocharis Morsus-ranae* . . . . . 0·267
36. *Solanum tuberosum* . . . . . 8·163
37. *Nicotiana affinis* . . . . . 7·524
38. *Lycopersicum esculentum* . . . . . 6·569

We need not here describe the method by which the numbers were obtained, but will only remark that they are strictly comparable. They really represent *the amount of maltose in grams which the diastase of ten grams of leaf is able to produce by hydrolysis from soluble starch in forty-eight hours at a temperature of* 30° C.

As an example of the accuracy of which the method is susceptible, we give the results of two determinations of diastase on the same sample of *Tropaeolum*-leaf:—

(1) 4·96
(2) 4·90.

We see from the table that the foliage-leaves of all plants examined contain more or less diastase, but that the amount of enzyme varies enormously in different plants, and, within narrower limits, even in the same plant at different times.

It will help us to realize the high diastatic power of some leaves if we compare them in this respect with an ordinary *malt*, acting under the same conditions. We see that the leaves of *Pisum sativum* contain a sufficiency of diastase to convert twenty-four times their own dry weight of starch under the standard conditions. Under these same conditions an ordinary pale barley-malt will convert sixty-three times its own weight of starch, so that we are led to the remarkable conclusion that the diastatic activity of the leaf of *Pisum sativum* is, weight for weight, between one-half and one-third of that of an average barley-malt.

At the other end of the scale as regards diastatic activity, we have the leaves of *Hydrocharis Morsus-ranae*, which can only hydrolyze 2·5 per cent. of their own dry weight of starch, but there is no doubt that in this case our method gives too low an expression of the diastatic activity owing to the large quantity of tannin which the leaf contains, and which partially inhibits the action of the diastase.

It will be noticed in the table that the Leguminosae stand out pre-eminently for the high diastatic power of their leaves. The one exception is the Lupin, and here again we have to do with a leaf containing a good deal of tannin.

In considering the very variable diastatic function of the leaves of plants it is a matter of considerable interest to determine if there is any correlation between the amount of diastase in the leaf, and the facility with which the particular leaf forms starch in its chloroplasts.

Unfortunately we do not at present possess a sufficient number of accurate observations on the starch-production of various plants to determine this point with certainty. We are not however without some data to guide us.

A. Meyer[1] has generalized his observations on the facility of starch-production in leaves by arranging the Natural Orders of the Dicotyledons which he has examined in five different classes, according to the amount of starch which they can produce in their leaves under favourable conditions. In the first class he places the Solanaceae and the Leguminosae as being able to form large quantities of starch. As already shown, the Leguminosae are highly diastatic, pre-eminently so in fact, and the three Solanaceae examined Nos. 36–38 come next to them in this respect.

Amongst the Monocotyledons it has been long known that certain of the Liliaceae form little or no starch in their leaves. The Liliaceous plants are certainly, according to our experience, very poor starch-formers, and it is interesting to note that they are also very poor in diastase, especially those species which never produce any starch at all. Of the three Liliaceous plants given in the table, Nos. 23–25, *Funckia sinensis* can produce a moderate amount of starch, whilst *Allium Cepa* and *Hemerocallis fulva* are not starch-producers. It is a noteworthy fact that the first-named plant is more diastatic than the two last.

Our observations certainly suggest that readiness of starch-production in the leaf under the action of light is related to the occurrence of diastase, and this fact alone renders it probable that the dissolution of the starch is in some way or other brought about by the enzyme.

That the diastatic function of leaves varies within certain limits in the same plant is evident from an inspection of Nos. 12–20 in the table, which show the amount of diastase met with in the leaves of *Tropaeolum* taken at different times.

We have endeavoured to ascertain if these fluctuations in diastase are in any way periodic, and if they are governed, like the fluctuations of starch in the leaf, by any external conditions to which the plant has been subjected.

[1] Bot. Zeitung, 1885, Nos. 27–32.

The results we have obtained in following up this inquiry are, we think, of considerable interest.

If the diastase is determined in a certain number of half-leaves taken during the day, and the other halves of these leaves are left still attached to the plant and are examined some hours after darkness has set in, we find that in the latter case, under conditions in which the leaf is being depleted of starch, the diastase increases very much in amount.

We give here the observed increases in diastase in the leaves of *Tropaeolum* under these conditions.

At 10 p.m. on an autumn evening there was 35·6 per cent. more diastase than there was at 5 o'clock in the afternoon.

In another case at 11 o'clock at night there was 70·3 per cent. more diastase in the leaf than at 3 o'clock in the afternoon.

In a third case at 5.30 in the morning there was 63·5 per cent. more diastase in the leaf than at 4 o'clock in the afternoon previous.

We find that the same increase in diastase takes place if the leaves are plucked and placed in darkness with their stalks in water. In leaves of *Tropaeolum*, so treated for eighteen hours, the diastase more than doubled in amount, whilst the starch had almost entirely disappeared.

No matter how the experiments were conducted, we were always led to the same result,—the conditions which are favourable for a decrease in the leaf-starch result in an increase of the leaf-diastase.

At first sight it seemed possible to explain this accumulation of diastase in darkness without having to fall back upon the supposition that the rate of production of the diastase by the living elements of the cell is variable. It is quite possible to imagine that the protoplasm of the assimilating cells secretes a certain definite amount of diastase which is constant under all conditions of insolation. As long as assimilation is proceeding with sufficient activity to maintain an excess of starch in the chloroplasts or amyloplasts this diastase is being used up in re-dissolving the starch, and consequently the enzyme does not accumulate in the cell. Supposing however that the plant is now placed in the dark, the starch diminishes and finally disappears, and there is consequently a diminished draft upon the diastase which now accumulates in the cell, not because the enzyme is being produced faster, but because less is being used up in a given time for hydrolysis.

There are, however, serious objections to this simple explanation. One of them is that the observed increase in diastase is out of all proportion to the starch actually dissolved. We should in fact expect a very much smaller increase in the diastase than is actually observed. Moreover this view necessarily postulates a constant formation and re-dissolution of starch as going on *simultaneously in the same cell*, a condition of things very unlikely to occur, for it is very difficult to imagine that solid starch can be deposited from some such pre-existent form as soluble-starch at a time when the cell contains far more than a sufficiency of diastase to hydrolyze that starch completely. Our own observations on the leaf of *Hydrocharis* are altogether opposed to the view that formation and dissolution of starch can take place simultaneously in one and the same cell.

A more probable explanation of the increase of leaf-diastase in darkness was suggested by some observations which we have described in an earlier paper on the Germination of the Grasses[1]. We there showed that the epithelium of the scutellum must be regarded as a specialized tissue for the secretion of diastase by the embryo of the Grasses, and we also showed that this power of diastase-secretion is inhibited in a remarkable manner as long as the embryo is artificially supplied with solutions of certain sugars, and is therefore not obliged to obtain its nourishment from the starchy reserve-materials of the endosperm. The secretory function of the epithelium is in fact only exercised when the embryo is in danger of starvation.

We find that a somewhat similar explanation is applicable to the increased production of diastase in leaves when these are placed in the dark.

As long as the conditions are favourable for assimilation, the leaf-cells are necessarily supplied with an abundance of newly assimilated materials in the form of sugars, more in fact than can be easily made use of or translocated. The excess of nutritive material is in part deposited as starch.

At this period there is little or no elaboration of diastase by the cell-protoplasm, probably none at all in those cells in which starch-deposition is actually going on.

When the light fails and assimilation consequently falls off, the living cells speedily use up or translocate the excess of assimilated

[1] Journ. Chem. Soc., vol. lvii. p. 458, 1890.

products, such as cane-sugar, and begin to draw their supplies from the more permanent starch. To enable the cells to do this effectually, the somewhat starved protoplasm now commences to elaborate the needed diastase more rapidly, and the secretion of the enzyme becomes accelerated as the starvation-point of the cell is reached.

According to this view the secretion of diastase by the leaf-cell is, like that of the embryo of the Grasses, to some extent a *starvation-phenomenon.*

We have been able to bring forward a considerable amount of experimental evidence in favour of this view.

We know, from the experiments of Boehm[1], and of Arthur Meyer[2], experiments which we have ourselves frequently verified, that the assimilating cells of leaves can form starch in their chloroplasts, even in the absence of light, providing the leaf is artificially supplied with a sufficiency of soluble carbohydrates, such as cane-sugar. The sugar thus artificially supplied is metabolized by the cell in exactly the same manner as if it were a direct product of assimilation.

Now we find, if leaves are in one case supplied artificially with a sugar-solution, and in the other case are allowed to deplete in water, that in a very short time the starved leaves, the cells of which are going through a process of autophagy, contain very much more diastase than do those which have been carrying on their metabolic processes, and in fact storing up starch, at the expense of the sugars supplied.

These results all tend to show that the 'starvation-hypothesis' is probably correct as regards foliage-leaves as well as germinating seeds, and that the periodic fluctuations of diastase which we know take place in plants are indirectly connected with the amount of nutriment supplied to the leaf-cells by the assimilatory processes.

The production of diastase in the leaf, like that of starch, is connected with the action of light, but it is manifest that the conditions which favour the production of starch are just those which inhibit, or tend to inhibit, the production of diastase, and vice versa.

We have examined very carefully into Wortmann's statement that leaf-diastase, when it does occur in the plant, cannot act upon the solid starch-granule. This statement is certainly not strictly correct, and we have given in our paper ample proof of this. Some starches,

[1] Bot. Zeitung, 1883, Nos. 33, 49.
[2] Bot. Zeitung, 1886, Nos. 5–8.

especially buckwheat-starch, are very readily acted on by the leaf-diastase of a plant like *Pisum sativum*, and even wheat-starch and barley-starch are slowly acted upon. In the case of buckwheat-starch it is not difficult to obtain considerable action within two hours of the commencement of the experiment. In fact this starch can be attacked by the diastase of some leaves with a rapidity approximating that under which the starch is observed to disappear in the living leaf-tissue.

In his contention that the starch of leaves only disappears under the action of living protoplasm, and is not conditioned by any enzyme-action, Wortmann relies, not only upon the supposed rarity of diastase in leaves, but also upon the alleged impossibility of procuring the dissolution of starch in the leaf when the plants are placed in an atmosphere of carbon dioxide, or when the free respiration of the leaf cell is interfered with in any other way.

It seemed to us however that under the conditions of Wortmann's experiments little or no disappearance of starch could be expected to take place in the leaf, even if its dissolution depended upon the simple chemical function of a portion of the cell-contents.

There seemed to be a much better chance of obtaining solution of starch in the leaf-tissue under the action of its own diastase if the leaf could be completely killed without destroying or inhibiting the action of the contained enzyme. The products of hydrolysis could then readily pass outwards when the physical conditions for diffusion were favourable.

With this object in view the leaves were killed by immersion for some time in an atmosphere charged with chloroform vapour, and were then placed under the most likely conditions for dissolving the starch under the action of the leaf-diastase.

Although numerous trials were made with leaves of *Tropaeolum*, *Hydrocharis*, and *Pisum sativum*, by half-leaf methods, we have never succeeded in satisfactorily demonstrating dissolution of starch within the cells of the killed leaf, not even if already partially depleted leaves were taken, in which the diastase was at a maximum.

Apart entirely from any metabolic function of the cell, it is evident that the starch-grains embedded in the chloroplast of a living leaf-cell must, whilst still surrounded and carried along by the streaming protoplasm, be placed under conditions far more favourable to the action of any enzyme the cell may contain, than is the case when all

motion of the protoplasm has been arrested, either by limitation of oxygen or by chloroform-treatment.

In the case of a cell the vitality of which is arrested or destroyed, the enzyme, if it is to reach the starch at all, must do so by a process of diffusion through the protoplasm, and more or less of the chloroplast itself. Now it seemed quite possible, in lack of evidence to the contrary, that the negative results we had so far obtained with killed leaves might, after all, be due to the inability of the highly colloidal diastase to gain ready access to the starch by diffusion.

As no determination of the diffusion-rate of diastase had been made, it was necessary to do this in order to see how much value has to be placed on this objection.

This was done in the following manner:—

A 3 per cent. gelatine-solution, when just on the point of setting, was mixed with a little solid buckwheat-starch which remained suspended in the solidified gelatine. On the top of this mixture, which was contained in a small beaker or other suitable vessel, there was then run a 3 per cent. solution of gelatine containing diastase. When this had perfectly set, we had the starch-gelatine below and the diastase-gelatine above with a perfectly sharp line of separation between the two. After a suitable time the gelatine-mixture was made as hard as possible by immersion in a freezing mixture and was then divided into vertical slices for examination with the microscope. The extent of the diffusion of the diastase from the upper into the lower layer was indicated by the distance through which action on the starch-granules could be detected.

In the case of the diastase of malt-extract the diffusion of the enzyme went on for several days at a very uniform rate of 0·145 mm. per hour, whilst precipitated and re-dissolved diastase diffused at only about one half of this rate, viz. 0·061 mm. per hour.

Now if we consider the size of a palisade-cell of the leaf of a *Tropaeolum*, which is about 0·1 mm. long, and 0·025 mm. in width, we see very clearly that, if the rate of diffusion of diastase through dead protoplasm is anything like it is in gelatine-jelly, the non-disappearance of starch in a chloroformed leaf cannot be due to any inability of the diastase to reach the starch by the ordinary process of diffusion.

Taking all the evidence which we have been able to collect into consideration we are compelled to admit that the *first stage* of dissolution of the starch-granule in the leaf is in some way or other

bound up with the *life* of the cell. When however we review all the facts, and give due weight to (1) the constant and abundant occurrence of diastase in leaves, (2) to the apparent correlation of this diastase with the occurrence of starch, (3) to the remarkable periodicity of the rise and fall of diastase, and (4) to the correlation of this periodicity with the appearance and disappearance of starch; we cannot possibly accept Wortmann's view that the dissolution of starch in the leaf is in no way conditioned by a starch-dissolving enzyme.

In fact we believe that our experiments, when duly considered, will establish beyond all doubt the physiological importance of diastase as an active agent in the dissolution and translocation of starch, not only in leaves but also in the growing parts of all plants.

It is manifest that if diastase plays such an important part in the breaking down the starch, it is a necessary corollary that *maltose* should be found amongst the sugars of a leaf from which the starch is disappearing. That such is actually the case is rendered evident from a review of the results we have recorded in the concluding part of our paper dealing with the *sugars of the leaf*.

As there seemed to be every probability of the sugars of the foliage-leaves of different plants varying considerably we thought there would be a better chance of arriving at some conclusions as to the genetic relations of the various sugars to each other and to the starch, if we confined our attention to the leaves of a single species of plant. After several trials we selected *Tropaeolum* as the plant most suitable for experiment, especially as we had already subjected it to full examination in other directions.

Our experiments were planned with a view to ascertain not only the nature of the leaf-sugars and the variations in amount and relative proportions at different times, but also in the hope that we should throw some light on the relation which each sugar bears to the primary assimilation-products on the one hand, and to the leaf-starch on the other. We hoped in fact to determine which are the true 'up-grade' sugars towards starch, and to see if there is any evidence of the existence of 'down-grade' sugars proceeding from the hydrolysis of starch, and its subsequent metabolism.

It is manifest that the presence or absence of *maltose* must have a very important influence upon our ideas of the physiological mechanism involved in the dissolution of starch in the living cell, for it is scarcely probable, if the disappearance of the starch is brought

about by the living elements of the cell only, that the metabolized products would be identical with those of mere hydrolysis under enzyme action.

Another point we had in view was, by observations on the 'down-grade' sugars, to determine if these afforded any evidence for or against the supposition of Sachs that the starch is in a continual and rapid state of flux at all times, and that the products of assimilation necessarily pass through the form of starch during their metabolism.

It was also expected that some light would be thrown on the particular form in which the sugars wander from cell to cell in their passage out of the leaf into the stem, and on the nature of the sugars which contribute the most readily to the processes of respiration and new cell-growth.

The only sugars which we have been able to find in the leaves of *Tropaeolum* are *cane-sugar*, *dextrose*, *levulose*, and *maltose*. No pentoses are present, or at most but mere traces.

The total amount of the sugars, like that of starch, is subject to great variations, and the relative proportion which the sugars bear to each other also varies very much.

We have in the full paper described in detail the methods we employed for isolating and quantitatively determining these sugars.

As an example of the concordant results which can be obtained we give two determinations made upon the same sample of *Tropaeolum*-leaves.

	(B) per cent.	(A) per cent.	
Cane-sugar . .	3·24	3·39	
Dextrose }	4·69	2·41	4·59
Levulose }		2·18	
Maltose . . .	0·76	0·61	
Total sugars per cent.	8·69	8·59	

We have obtained a considerable amount of information from the systematic examination of the sugars and starch contained in leaves which have been placed under determinate conditions of illumination, both when still attached to the plant and after separation from it. We give one or two examples of these analyses.

The following were the results obtained by an examination of leaves separated from the plant, A. at 9 a.m., and B. at 4 p.m. of a summer's day:—

Series I.

	A. (9 a.m.) per cent.	B. (4 p.m.) per cent.
Starch	3·24	4·22
Sugars—		
Cane-sugar . .	4·94	8·02
Dextrose . . .	0·81	0·00
Levulose . . .	4·78	1·57
Maltose . . .	1·21	3·62
Total sugars per cent.	11·74	13·21

We have already seen that when leaves are plucked and placed exposed to sunlight with their stalks in water there is a large increase in weight of a given area of the leaf-lamina, owing to the accumulation of the products of assimilation. The following results show the varying proportions of starch and the different sugars under these conditions:—

A. Leaves plucked at 5 a.m.

B. Leaves plucked at 5 a.m. and insolated apart from the plant until 5 p.m.

Series II.

	A. per cent.	B. per cent.
Starch	1·23	3·91
Sugars—		
Cane-sugar . .	4·65	8·85
Dextrose . . .	0·97	1·20
Levulose . . .	2·99	6·44
Maltose . . .	1·18	0·69
Total sugars per cent.	9·69	17·18

We have also investigated the changes which go on in the carbohydrates when leaves are plucked and placed in the dark. The following is an example of such an experiment:—

A. Leaves plucked at 3 p.m.

B. Leaves plucked at 3 p.m. and placed in the dark for 24 hours.

Series III.

	A. per cent.	B. per cent.
Starch . . .	3·693	2·980

	A. per cent.	B. per cent.
Sugars—		
Cane-sugar . .	9·98	3·49
Dextrose . . .	0·00	0·58
Levulose . . .	1·41	3·46
Maltose . . .	2·25	1·86
Total sugars per cent.	13·64	9·39

By the constant repetition of experiments such as those mentioned we have been able to arrive at certain definite conclusions of importance.

In the first place, our experiments are decidedly opposed to the view that either dextrose or levulose is the first sugar formed by assimilation, at any rate in the leaves of *Tropaeolum*. They point, however, to *cane-sugar* as being the first distinctly recognizable sugar to be synthesized by the assimilatory processes.

There seems every reason to believe that this cane-sugar, which may be regarded as the starting-point of all the metabolic changes taking place in the leaf, accumulates in the cell-sap of the leaf-parenchyma when assimilation is proceeding vigorously. When the concentration exceeds a certain point, starch commences to be elaborated by the chloroplasts at the expense of the cane-sugar. This starch forms a somewhat more stable reserve substance than the cane-sugar, and is only drawn upon when the more readily metabolized cane-sugar has been partially used up.

That the starch which is formed in the chloroplasts is, strictly speaking, not autochthonous, but owes its origin to antecedent cane-sugar, seemed probable, not only from a consideration of the results we have described in the paper, but also from Boehm's and Meyer's experiments on the artificial nutrition of leaves by solutions of certain carbohydrates, and also from results which we obtained and described in 1890 on the starch-producing powers of some of the sugars when used as a nutrient for embryos of the Grasses. In all these cases it was found that cane-sugar surpasses all other carbohydrates in starch forming.

As regards the particular form in which the carbohydrates wander from cell to cell in the leaf and finally enter the stem, we think our experiments warrant us in the statement that the cane-sugar is translocated as dextrose and levulose, and the starch as maltose,

but this latter translocation only occurs when the partial starvation of the cell has rendered possible the dissolution of starch by enzyme-action.

From the invert-sugar, derived from the cane-sugar, the dextrose is more readily used up for the respiratory processes, and possibly also for new tissue-building, than is the levulose: hence in a given time more levulose than dextrose must pass out of the leaf into the stem.

Knowing as we do how enormous is the resistance which living protoplasm affords to the ordinary physical processes of diffusion, it seems highly improbable that the wandering of the sugars in living plant-tissue is altogether dependent upon osmosis. It is no doubt to the continuity of the protoplasm from cell to cell, which may now be regarded as an established fact, that we must look for a full explanation of those rapid translocations of certain substances which we know take place. That diffusibility is however a determining factor of importance cannot, we think, be doubted when we regard the nature of the substances which up to the present time have been recognized as wandering metabolites.

THE GENUS TREMATOCARPUS.—With reference to my note on this genus in 'Annals of Botany,' vol. vi, no. 21, April, 1892, a letter has been received from Dr. Zahlbruckner, of which the following is a translation.

'In the "Annalen des k. k. naturhist. Hofmuseums in Wien," vol. vi, I described my genus *Trematocarpus*, basing it on *Lobelia macrostachys*, Hook. et Arn., which differs absolutely from all species of *Lobelia* in the structure of its fruit. Mr. W. B. Hemsley raised objections, in the 'Annals of Botany' of April, 1892, to my proposed new genus on the ground either that the genus *Trematocarpus* does not refer to *Lobelia macrostachys*, Hook. et Arn.; or—and this seems to be regarded by Mr. Hemsley as more probable—that the capsules in my possession were not normal, but had been eaten by insects. As to the latter objection, I can only state that the capsules on which my description is based are quite normal, and that my description is an accurate account of the facts. Were the holes in the wall of the capsule due to insects, the margins of the holes would consist merely of the tissue of the wall, or possibly there might have been some development of cork from callus. But this is not the case. On the contrary, the holes are bounded by a raised ring consisting of fibrous

sclerenchyma. Moreover, the apex of the woody capsule is and remains completely closed: how then can the dissemination of the ripe seeds be effected? I hope that Mr. Hemsley—to whom I have since sent a photograph of Wawra's plant and a ripe capsule—will be able to confirm my description of the capsule in every detail.

The first objection demanded careful reconsideration of the question whether or not the plant were really identical with Hooker and Arnott's species: for, in view of the inadequate descriptions of the authors, there was a possibility that I might have been misled in spite of conscientious investigation. I addressed myself to Mr. Hemsley for further information, and he was so good as to send me a capsule of the original species. This material made the matter quite clear to me. It was apparent to me in a moment that Mr. Hemsley had before him only quite young unripe fruits of the original species. Capsules in this stage of development are present also in the upper part of the inflorescence of Wawra's plant, and I have also seen them on a plant collected by Hillebrand. This is the cause of the error into which Mr. Hemsley, like Hillebrand, has fallen. Mr. Hemsley, since seeing the photograph which I sent him, has written to me saying that Wawra's plant is undoubtedly identical with *Lobelia macrostachys*, Hook. et Arn.

I conclude, therefore, that there can no longer be any doubt that (1) the creation of the genus *Trematocarpus* was fully justified; and that (2) *Trematocarpus* refers to *Lobelia macrostachys*, Hook. et Arn.

In conclusion, I would merely point out that *Trematocarpus* is not at all closely allied to the genus *Lobelia*, and that I propose to state the reasons for this assertion in a paper which will appear shortly.'

Vienna, Jan. 18, 1893. DR. A. ZAHLBRUCKNER.

In reply, I have only time at present to state that I have again superficially examined the Kew specimens of *Lobelia macrostachys*, but I am still of opinion that the orifices in the ripe capsule are not pores of dehiscence in the ordinary acceptation of the term, because they either appear irregularly on any part of the capsule and vary in number from one to several, or, what is more frequent, there may be none at all. However, my colleague, Dr. Stapf, has undertaken to investigate the anatomy of the capsule: the conclusions at which he may arrive will be published in the next number of the 'Annals of Botany.'

Kew. W. BOTTING HEMSLEY.

On the Structure of the Haustoria of some Phanerogamic Parasites.

BY

GEORGE J. PEIRCE, S. B.

With Plates XIII, XIV, and XV.

THE work, the results of which are given in the following pages, was undertaken at the suggestion of Professor Eduard Strasburger, and performed under his supervision. Five species of *Cuscuta* have been studied. The material of *Cuscuta americana*, L., was collected by Dr. Fr. Johow in 1883 on the Island of Grenada in the West Indies. It consisted of well-grown specimens of the parasite, some in flower, others younger, others older, and considerable quantities of the host-plants, all preserved in strong alcohol and in excellent condition for histological study. The material of three of the other species, *C. epilinum*, Weihe, *C. epithymum*, L., *C. glomerata*, Choisy, was grown in the Botanic Garden of the University at Bonn and preserved in alcohol. *C. europaea*, L., the last species studied, was found growing wild, and was also preserved in strong alcohol. I regret to say that all the material of the same species was collected at one time, and that for this reason interesting questions as to the changes which may take place in these parasites growing in temperate regions, as the season when life is possible draws to a close, cannot now be answered; but I hope in a subsequent paper

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to add, to the facts now set forth, observations on other species of *Cuscuta* collected and 'fixed' at different times during summer and autumn.

Cuscuta americana, L.

Cuscuta americana, L. finds its nourishment on a variety of herbaceous and woody plants growing in the West India and Bahama Islands. The host-plants at my disposal were two species of Mimoseae, one of Apocynaceae, and *Thevetia neriifolia*, Juss. I regret that the species of only one of these was determined, or is determinable. The structural differences between the hosts induce certain interesting differences in the growth of the haustoria, as will be shown further on. The parasite displays a remarkable difference in size between those parts which apply themselves to a host and those which are far removed from the sources of food. Thus, the average diameter of a well-nourished plant, in a region whence numerous haustoria penetrate a host, is 2–3 millimeters, while the average diameter of parts several centimeters distant from active haustoria is only 1 millimeter or less. I do not venture to say positively, owing to the incompleteness of the material at hand, that when two or more stems twine about one another instead of about a host, haustoria are never developed; yet in one case observed, where three stems twined together for a distance of several centimeters, no haustoria were developed. If, however, one stem touches a host and sends haustoria into it, those which are twining with it quickly develop haustoria which, if not able to reach the host, strike into that stem which is being nourished as well as mechanically supported by the foster-plant. If, then, such be the general rule, it is evident that not contact merely is sufficient to induce the development of haustoria.

When a *Cuscuta*-stem passes from one part of the host to another part, in such fashion that some haustoria if they develop far must strike only into the air (as when the parasite grows directly across from a branch to a leaf, or vice versa), these haustoria, having nearly reached the surface of the

mother-stem, develop no farther, remain abortive, and are made evident only by swellings of greater or less size. The epidermal and cortical tissues have been pushed out by the subjacent growing haustoria, and though the epidermal cells may have begun to differentiate in the way to be described further on, they do not greatly differ from their neighbours, and furnish an unbroken and normally cutinized protective covering. In these abortive haustoria no differentiation of tissues takes place, the cells remaining parenchymatous. Such a haustorium, early aborted, is shown in transverse section by Fig. 2′, Pl. XIII.

Cuscuta americana, as shown by Fig. 1, twines from left to right around its host in a spiral sometimes short and close, oftener long and loose. The outline of the haustorium as seen in superficial view (see Fig. 1, *c*, *d*, Pl. XIII) is elliptical. The long axis of this ellipse is not parallel with the axis of the parasite, but is approximately parallel with the long axis of the host. According as the spiral formed by the parasite in twining about its host is steep or gradual, the long axis of the ellipse is at a lower or higher angle with the long axis of the parasite. In this way the conducting elements of the haustorium are so oriented as to be in the best possible position for uniting with the corresponding elements in the host.

The form and structure of a young stem two or three centimeters above the youngest haustorium is shown by the cross-section in Fig. 2. The outline is approximately round, with more or less pronounced lobes. The epidermis, seldom if ever interrupted by stomata and bearing no trichomes, consists of a single layer of rather small cells, well supplied with protoplasm, whose outer walls are thickened and cutinized. Between the epidermis and the pericycle is a parenchymatous tissue whose cells are of two sorts. About midway between the pericycle and the periphery is a more or less wavy and somewhat broken line composed of small, irregular, rather compressed cells. Their irregularity is due to the torsion of the stem. The rest of the cortex consists of large,

thin-walled cells with fairly abundant protoplasm. Small intercellular spaces are numerous. Just within the pericycle one finds a somewhat irregular ring of lactiferous tubes, large, thick-walled, and angular in cross-section. The small fibro-vascular bundles are separated from one another by masses of parenchyma-cells like those which compose the pith, large and thin-walled. The fibro-vascular bundles are of the collateral open type, but with more phloëm than xylem. The phloëm consists of soft bast only, no hard bast or sclerenchyma-fibres ever being developed in the stem. The xylem consists of ducts and wood-cells. The ducts earliest formed are soon resorbed, leaving a large air-space. The cambium may always be clearly seen.

In a radial section (see Fig. 3) through a bundle, one sees that the bundles are sharply marked off from the fundamental tissue in the centre of the stem by the large air-space (*a*); that the ducts (*d*), either reticulated or more commonly pitted, are few in number; that the cambium-cells (*c*) are not so long as the elements developed from them; that the long sieve-cells form tubes of very considerable diameter (*s*), and on their horizontal walls thick callus-plates are formed; that the companion cells (*c.c*) are sometimes longer than the elements of the sieve-tubes, and have more or less oblique terminal walls; that all the elements of the bundle are thin-walled.

Making now a radial section in the region just above the youngest haustorium, the new haustorium is found to originate in the cortical parenchyma just beyond the pericycle, in a longitudinal line of cells which, by rapid successive divisions in a tangential direction, soon form a mass of the shape of a plano-convex lens, whose convexity is toward the periphery of the stem. By the growth and division of these cells the structure assumes a conical shape (see Fig. 4), the cells at the base being long, those toward the apex becoming smaller, until the minimum is reached in one or two rows from the tip. The tip consists of long, rather narrow cells. The two or three rows immediately behind the tip are made up of

the cells by the repeated divisions of which the conical haustorium increases in length. The haustorium consists of these cells and their offspring only, no others from the cortical parenchyma or the epidermis contributing at any time to its mass.

By the growth of the haustorium, the cells of the cortical parenchyma which immediately overlie it become compressed and, pushing outward, form a slight elevation on the surface of the stem. Meanwhile the cells of the epidermis covering this region of growth and consequent pressure, and those in the near vicinity, again begin to grow. Their outer cutinized and thickened walls are partially resorbed. These rejuvenated epidermal cells grow out into long thin-walled papillae, with abundant protoplasmic contents, and large nuclei situated near the tips of the cells (see Fig. 4′). In this way a cushion-like structure is formed (see Figs. 4 and 5), highest at the centre and at the periphery, with a broad shallow depression between; and by the circumnutation of the stem it becomes closely applied to the surface of the host. The raised margin of the sucker acts more or less as a prehensile organ, as already pointed out by Chatin in his description of *C. monogyna*, Vahl[1].

The epidermal cells composing the cushion, being now applied to the epidermal cells of the host, exude through their thin walls a solvent which attacks and dissolves the walls and contents, first of the epidermal, then of the immediately underlying cortical cells, of the host. Thus an opening into the host is effected. This solvent activity is greater in the centre of the cushion, directly over the haustorium, and diminishes rapidly towards, and ceases altogether before reaching the periphery. During this time the haustorium, already nourished perhaps by the dissolved cells of the host, grows rapidly, pushes its way by the combined action of solution and pressure through the overlying cortical parenchyma and, breaking through and crushing the epidermis which covers it, passes into the host through the opening

[1] A. Chatin, Anatomie Comp. des Végétaux Parasites, Dicotyledones. Paris, 1857-1862.

made by the cushion-cells, carrying with it along its sides for a short distance into the host some of the cortical and epidermal cells which overlaid it. It completely fills this opening, enlarging it by solution, and later by pressure, thus soon forming around itself a mass of compacted walls of dead cells from which all nutritive matters have been removed by solution (see Figs. 6 and 7).

The long cells forming the apex of the haustorium have already begun to grow rapidly, their walls, especially at the tips, remaining thin. They dissolve their way through the cortical tissues of the host, exercising little if any pressure, as the absence of collapsed cells in front of them shows. (To save useless repetition, the reader is referred to pages 307 and 308 where the relations of these cells with those of the host are discussed in detail in the description of *C. glomerata*). Immediately behind these cells, which have now become papillate, and may conveniently be termed collectively the *sucker*, other cells by continued divisions provide for the elongation of the haustorium as fast as room is made for it in the host by the action of the sucker.

The subsequent extension of the haustorium varies in accordance with the structure of the host. If the host be a plant similar in structure to the parasite, that is, with a single ring of open collateral bundles separated by considerable masses of parenchyma, the haustorium grows generally through the cortical into the interfascicular parenchyma. If the fibro-vascular bundles are widely separated by parenchyma, the haustorium approaches and applies itself laterally to the bundle nearest to it. If the scattered bundles are not far apart, so that the haustorium occupies nearly all the space between them, it grows laterally more rapidly than in the apical direction, and so applies itself to both bundles (see Fig. 6). This is the case in one of the Mimoseae studied. Having applied itself to one or both of the bundles between which it grows, the haustorium grows but slowly at its tip, continuing however to extend itself for a greater or less distance into the pith. If the host

(see Fig. 8) consist of a stem in which, instead of a regular ring of scattered bundles uniform in size, the ring be wavy, with large bundles on the crests and single small bundles in the rather deep depressions of the ring, the haustorium, having applied itself laterally to one or two bundles of adjacent crests and continuing to grow toward the centre of the host, will come directly upon the smaller bundle in the depression. Instead of dissolving its way into and through this bundle, the tip divides into two parts which pass on opposite sides of the bundle, with which they both presently unite. In other words, the fibro-vascular bundle in the depression of the general ring divides the haustorium like a wedge, and the haustorium, instead of uniting with one or two, unites with three fibro-vascular bundles and destroys none. The advantage to the parasite of this arrangement is evident. Such is the case of the undetermined Apocynaceous host in Dr. Johow's collection, as shown by Fig. 8.

The other species of Mimoseae and *Thevetia neriifolia* show still a third mode of growth. In these the fibro-vascular bundles soon become confluent, first in their xylem area, later in the soft bast. The hard bast masses in *Thevetia* remain distinct. In the *Mimosa* a sclerenchyma-ring takes the place of the hard bast. As the haustorium grows into these plants, it meets in the *Mimosa*, and may meet in *Thevetia*, a strongly lignified mass of thick-walled cells. These, however, resist the solvent action of the haustorial cells only for a short time, and the sucker applies itself to the ring of soft bast. This it more quickly penetrates, and finally attacks the wood-elements which in both hosts are closely compacted, thick-walled, and thoroughly lignified. These it dissolves much more slowly and, depending upon the age and thickness of the wood, more or less completely (see Fig. 9). It is seldom that the haustorium reaches the pith in the *Mimosa*; it does sometimes in *Thevetia*. The parasites, in uniting with the conducting tissues of these two hosts, do so by destroying part of the conducting tissues. The vitality of the hosts is thereby considerably

impaired, and they are correspondingly less nutritious to the parasites. It is noticeable that, so far as the material at hand is concerned, the parasites living on these plants are smaller, and produce fewer flowers, than those growing on the other two species. There may be reasons other than the structure of the hosts, and the consequent mode of growth of the haustoria, for the weakness of the parasites. The other reasons are unknown; this reason is evident.

While the sucker has been making its way through the parenchymatous tissues of the host to the fibro-vascular bundles, and while the rest of the haustorium has grown at the same rate with it, certain changes have been taking place in the shaft of the haustorium and in the parasite itself. If one makes a tangential section of a *Cuscuta*-stem so that it shall pass through a haustorium already imbedded at its distal end in the tissues of a host, at right angles with the long axis of the haustorium, one finds that the shaft of the haustorium is no longer composed of parenchymatous tissue only. If the section be made near the surface of the *Cuscuta*-stem it is seen that the haustorium consists of two concentric layers of tissue which are unequal in thickness (see Fig. 10). The outer or cortical layer is composed of fairly compact, parenchymatous, angular cells. This cortex abuts, without the interposition of a sharply-defined epidermis, upon the loose cortical parenchyma of the mother-plant, those cells immediately surrounding the haustorium being more or less compressed by its growth. Within this zone of cortex, and about one half as thick, is the central cylinder, composed at first of procambium-cells. These more or less rapidly and completely differentiate into three or four, sometimes more, fibro-vascular bundles, at first separate, but later confluent. The central procambium-cells become large, thick-walled, lignified, evidently ducts or tracheids (see Fig. 11, *t*). One or two layers of cells on either side of these remain merismatic for a time, and form, therefore, a temporary cambium (Fig. 11, *c*). Immediately outside these two cambium-layers are two masses of soft bast, consisting of cells of large and small diameter in varying proportions (Fig. 11, *p*).

These phloëm-layers are separated from the cortex by one or two layers of parenchyma-cells, and by the pericycle. We have therefore in the central cylinder of the haustorium several bicollateral fibro-vascular bundles which later become confluent, consisting of a central xylem, two cambiums, and two phloëms. The cambiums add not more than one row of cells to each side of the xylem, and two rows of cells to each phloëm area. They then cease to divide further, and retain their typical form and structure.

If now a cross-section of *Cuscuta*-stem be made through a haustorium of about the same age, we obtain a radial section of the haustorium which will enable us to complete our knowledge of its structure. We find, in the centre, the thick-walled lignified cells of the xylem. These are separated from one another by cross-walls, which are slightly, if at all, oblique. These cross-walls remain intact. Hence, the xylem is composed of tracheids only. The cross as well as the lateral walls of the tracheids are deeply pitted or reticulated. I have observed no spiral or annular markings. Bounding the xylem on either side are the two cambium or, if the structure be older, cambiform layers rich in protoplasm. Beyond these are the two phloëms consisting of sieve-tubes and their companion-cells. If the section be one of a haustorium of sufficient age, staining with an aqueous solution of aniline blue will demonstrate the callus-plates in the sieve-tubes, as will be described further on. The rest of the central cylinder is composed of elongated parenchyma-cells abutting, with the pericycle between, on the cortex. The differentiation in the haustorium begins near the base and extends towards its apex.

The same cross-section of the parasite will show that the haustorium, growing through the cortical parenchyma, is about opposite an interfascicular mass of parenchyma. The cells of this interfascicular parenchymatous mass, which are adjacent to two fibro-vascular bundles, begin to differentiate in two ways along two lines on each side. Running toward the base of the haustorium, from the xylems of the two bundles, one finds two rows of cells of considerable diameter (see Fig. 12 *t*,

where, owing to the plane of the section, only one row is shown) whose walls are being unequally thickened, leaving pits or reticulations. The differentiation extends through the pericycle to the base of the haustorium, the two rows gradually converging and uniting with its xylem. Thus the xylem of the haustorium becomes connected with the xylems of two fibro-vascular bundles in the mother-plant. In similar fashion two rows of cells, which, if large, divide by radial walls, run from the two phloëms of the same two axial bundles of the parasite to the two phloëms of the haustorium, and become differentiated into sieve-tubes and companion-cells (see Fig. 12, *s*, *s*). In sections old enough, callus-plates can be demonstrated in the sieve-tubes by the use of aniline-blue. Thus the phloëms of the haustorium are connected with the sieve-tubes of two fibro-vascular bundles in the mother-plant.

The question now presents itself.—Do the vascular tissues of the haustorium unite with corresponding tissues in the host? For convenience, a transverse section is made of the host where a tolerably old haustorium penetrates it. As is shown by Fig. 7, *t*, the xylem-elements of the haustorium apply themselves *directly* to the xylem-elements of a fibro-vascular bundle in the host; that is, those cells of the haustorium (consisting so near its apex of central cylinder mainly, with little or no cortex) which come by the solution of the intervening cells of the host into contact with the ducts, quickly differentiate into tracheids, the thick and thin places in their walls corresponding with the thick and thin places in the walls of the ducts to which they apply themselves. Thus the haustorial xylem connects a xylem-group of the host with two xylem-groups in the stem of the parasite.

By a similar process of solution of the intervening cells of the host the phloem-cells of the haustorium are united with the phloem-cells of the same bundle. That the union of haustorial sieve-elements with sieve-elements in the host is *direct* may more clearly be seen in a tangential section, such as is shown in Fig. 13, Plate XIV, for the contact of haustorial cells with the cells of the host is usually by their

radial walls. If one can demonstrate the presence of a callus-plate between two cells, no doubt can be entertained as to the nature of these cells; they are sieve-cells. If a tangential section of the host, passing through the sieve-tube area of a fibro-vascular bundle to which a haustorium had applied itself, be laid for a half-hour in a moderately strong aqueous solution of aniline-blue, then washed in distilled water to decolorize cellulose, lignified, and cutinized walls, and subsequently examined, in a clearing mixture of glycerine and water, under a high magnifying power, one finds the callus-plates of the sieve-tubes clearly defined by their blue colour. As is shown by Fig. 13, the haustorial tissues are easily distinguished from those of the host by the long axes of their cells being at right angles to those of the host. A careful examination will show that certain haustorial cells abut directly upon the sieve-tubes of the host, that the common wall between these haustorial cells and the sieve-cells is coloured along its whole face a faint blue, or that at certain places it is decidedly thickened and that these thicker parts are intensely blue (Fig. 13, *c*). When the wall is faint blue it is due to a thin deposit of callus over a considerable area; when the wall is deep blue in spots it is due to a thick deposit of callus in a limited area. One finds upon the radial walls between adjacent sieve-tubes of the host similar deposits of callus, extended and faintly coloured, or limited and deeply coloured. I have not seen such deposits on either side of the radial walls between sieve-cells and companion-cells. We know that callus is formed during the activity of sieve-cells as such, and not during their formation. Furthermore, by no means at present known can callus-plates be discovered in the youngest active sieve-cells. The deposit of callus is gradual and can be observed only after the cells have been active for some length of time. Between the sieve-cells of the host, and the adjacent haustorial cells, I have observed *thick* deposits of callus only on the side of the wall which limits the cavity of the sieve-cells of the host (Fig. 13, *c*). When, however, a thin deposit of callus is formed over

a considerable area, careful examination shows that the wall is stained on *both* sides. Thin sections, careful staining, decolorizing, and clearing, and high magnification are necessary to demonstrate this; yet with these precautions the results are positive. The companion-cells of the haustorial sieve-tubes unite directly with the companion-cells of the sieve-tubes of the foster-plant, as is to be expected from the direct union of the sieve-cells.

We see then that certain haustorial cells abut directly upon sieve-tubes in the host, and that callus-plates are formed between these. These haustorial cells are therefore sieve-cells. In a transverse section stained as above described, one sees that these sieve-cells of the haustorium which unite with the sieve-tubes of the host are the terminal elements of sieve-tubes which run through the haustorium to a stem-bundle of the parasite (see Fig. 7, Plate XIII). Thus, as the haustorial xylem connects a xylem of the host with two xylems in the stem of the parasite, so the haustorial phloëm connects a phloëm of the host with a phloëm in the stem of the parasite.

As has already been shown on pages 298–299, the central cylinder of the haustorium is made up of bi-collateral fibro-vascular bundles, at first separate, later confluent, consisting of one xylem and two phloëm-groups. When the proximity of the bundles of the host makes it possible for the haustorium to apply itself to two, as is generally the case in one of the Mimoseae, both phloëm-groups of the haustorium become active. In such cases the single strand of xylem divides into two near the tip, each of which, accompanied by phloëm, unites with one of the host bundles, xylem with xylem, phloëm with phloëm (see Fig. 6, Pl. XIII). Then the shaft of the haustorium consists for most of its length of bi-collateral bundles, but near the tip of twice as many collateral bundles. In the Apocynaceous host, as previously described (see Fig. 8, Pl. XIII), the haustorium usually unites with *three* bundles, thus demanding a division of the phloëm and a second division of the xylem. Since the third bundle with which the haustorium unites is small, and considerably

removed from the surface of the stem of the host, only a small amount of food is taken from it and the conducting tissues are weak. The xylem, already bifurcated in order to unite with the two larger outer bundles, sends a feeble strand of tracheids from each of the adjacent sides of the branches. Probably also two feeble strands of sieve-tubes and companion-cells are sent out from the two phloëms, but no callus-plates are shown by the aniline-blue test. In such cases the haustorium consists near its tip of two quite separate collateral bundles; a little further back of four such bundles; still further back of only two; and throughout the most of the shaft of only one bi-collateral large bundle. Where the haustorium impinges upon the ring of confluent fibro-vascular bundles in the other species of Mimoseae and in *Thevetia neriifolia* both phloëms are of course active and the xylem does not need to divide.

Having studied the origin, development, and structure of the haustorium we are ready to answer the question of its morphology. De Bary says[1]: 'Roots are found as lateral branches on members of their own kind, as well as on stems, rarely on leaves; some appear in definite morphological positions.' 'The invariably endogenous formation of lateral roots takes place in or close to vascular bundles or masses of wood or bast. Their vascular bundle is inserted directly and without branching on the nearest one of the main axis, or it divides into branches, which connect themselves with several bundles of the axis.' The typical primary bundles of roots, as is well known, are of the *radial* type, secondary changes causing the thickening roots to assume the structure of ordinary stems with collateral bundles. We have seen that the central cylinder of the haustorium consists at first of procambium surrounded by unmerismatic parenchyma; that this procambium changes in the centre into a xylem, and on either side of this into a phloëm; that parts of the procambium persist for a time as cambiums between the

[1] A. De Bary, Comp. Anat. of Phanerogams &c., p. 315. Clarendon Press, 1884.

xylem and the two phloëms; that there is never at any time a radial structure; and that its bi-collateral bundle is connected with two axial bundles of the mother stem. The haustorium, originating in the cortex near the pericycle from a definitely marked mass of cells, is formed from them by successive division. No other cells from the cortical parenchyma or the epidermis add themselves to it. This is clearly proved by the figures already described. The haustorium of *C. americana* differs decidedly, therefore, from the haustoria of *C. epilinum* and *C. epithymum* as described by L. Koch[1]. (I shall have occasion to refer later to the origin and structure of the haustorium of these two species.) We see then that the haustorium of *C. americana* is, so far as its origin shows, a lateral root; so far as the bi-collateral structure of its bundles is concerned it does not conform to the typical structure of a root. We must consider it to be morphologically a root, but that it has become modified in structure to do its work the better.

We come now to the morphology of the parts of the haustorium. The layer of cells forming the surface of the haustorium, though not differing greatly from those immediately underlying it, is sufficiently different, especially near the tip, to justify the name of epidermis being applied to it. In the young haustorium (see Fig. 5, *e*, Pl. XIII) it should receive the name of dermatogen. The cortex, consisting of several layers of cells at the base, is reduced to a single layer at the apex of the haustorium. It evidently originates from a periblem. What I have so far called the central cylinder plainly corresponds to the plerom. At the tip of the young haustorium these three layers can no more and no less be distinguished from one another than at the tips of most young roots. The growing-point of the haustorium corresponds in position (see Fig. 5, *g*, Pl. XIII) and in character with the growing-point of typical roots. It is covered by a single layer of cells which, in all but the youngest stages, I have called collectively the *Sucker*. But the sucker is merely

[1] L. Koch, Die Klee- und Flachs-seide. Heidelberg, 1880.

a part of the dermatogen, the only part which clearly differentiates. Its cells become papillate and perform the same functions as root-hairs; they absorb from the surrounding medium and, like root-hairs, exercise a solvent action upon what they came into contact with; but this solvent action is much more pronounced than that of ordinary root-hairs. The cells of the sucker are, then, physiologically root-hairs, but correspond in origin and position with the cap of ordinary roots. The haustorium is otherwise destitute of a cap.

The question presents itself—How was the bi-collateral bundle of the central-cylinder of the haustorium derived? On the facts already at hand only speculations can be based; but it may be hoped that in some *Cuscuta* the phylogeny of the bi-collateral structure will be shown.

To summarize the whole matter we may say that the haustorium of *C. americana* is morphologically a lateral root, since it originates endogenously and grows only at its tip; that it develops into a structure the bi-collateral vascular bundles of which are united with the fibro-vascular bundles of the mother-plant by two strands of tracheids and two strands of sieve-tubes and their companion-cells; that its xylem and phloëm unite directly with the xylem and phloëm of one or more bundles of the host; that an unbroken connection is made between the conducting tissues of the parasite and its host.

As to the distribution of the haustoria along the stem it is only necessary to say that they generally occur in groups; that these groups do not consist as a rule of more haustoria than there are bundles in the host; and that by the twining of the parasite successive haustoria are made to unite with different bundles of the host, thus insuring an abundant supply of food. It often happens that the food-supply exceeds the demand, and we thus find considerable quantities of starch deposited in the cortical parenchyma. Whether this is entirely or largely consumed in the processes of flowering and setting seed the material at hand is insufficient to determine.

CUSCUTA GLOMERATA, Choisy.

The plants of *Cuscuta glomerata* examined were growing luxuriantly on *Impatiens Balsamina*, L. at the time that they were 'fixed' and preserved in alcohol. Their stems are smoother and slightly larger in diameter than those of *C. americana*. They twine from left to right about the foster-plant in spirals generally shorter and closer than those formed by the preceding species. The haustoria are larger but otherwise differ only slightly in origin, form, and structure from those already described. They are oriented in conformity with the closer spiral made by the mother-plant. They consist of central cylinder and cortex, which latter is thickest at the base and is reduced to a single layer at the apex. The union of the conducting elements of the haustorium with the corresponding elements in a bundle or in two bundles of the host is here also direct, as shown by Fig. 14, Pl. XIV, and the connection between the conducting tissues of parasite and host is unbroken. Fig. 14, Pl. XIV, represents a section of a comparatively young haustorium which springs from a parasitic stem the long axis of which is inclined, as nearly as possible, at a right angle with the long axis of the plant about which it has twined; hence the union of the haustorial conducting elements with those of the mother-plant is only partially shown. In this figure is also shown in part the bifurcation of the strand of xylem near the tip of the haustorium in order to effect a union with the xylems of two adjacent bundles. Furthermore, one sees what less often occurs in *C. americana*, at least when growing on the hosts which I have examined, namely the encroachment of haustorial cells upon the cambium of the bundle attacked, and the union of haustorial xylem- and phloëm- cells in tangential, instead of radial, fashion with the xylem and phloëm-elements of the bundle of the host (Fig. 14, *t*).

Owing to its softer, less closely compacted structure, *Impatiens Balsamina* presents fewer mechanical difficulties to the study of the cells of the tip of the haustorium in their

relations to the cells which they attack, than do the more solid host-plants of *C. americana* which are at hand. That these relations are the same in both parasites will be shown further on. *C. glomerata*, like *C. americana*, strikes its haustoria into the leaves as well as into the branches of its host. The tissue of the leaf of *Impatiens Balsamina* is still more spongy than that of the stem, and in its loose parenchyma the bundles are rather widely separated. A haustorium which strikes into a leaf is less compact than one which penetrates a branch. The cells of the sucker, instead of growing as a cone straight forward, spread out into a brush-form. Some of those at the sides of the brush grow for some distance into the spongy mesophyll; the others unite ultimately with a fibro-vascular bundle. Fig. 15 shows the tip of a haustorium growing towards a bundle. The large, thin-walled, papillate cells dissolve the walls of the cells with which they come into contact, making perforations little larger than their own diameter. The walls of the parenchyma-cells are entirely dissolved only when enough haustorial cells intrude to occupy their whole diameter. The haustorial cells, as the figure shows, do not all grow at the same rate; hence the solution of the walls of opposing cells is a gradual process accomplished by several haustorial cells one after another. The contents of the opposing cells are dissolved rather slowly by the cells of the sucker, the starchy substances first, the protoplasmic later. The haustorial cells exercise little or no pressure, growing forward in the path which they have dissolved for themselves. Since the diameter of the haustorium is nearly the same from a point near its tip to its base, it occupies little more space than has been cleared for it by the solvent action of the cells of the sucker. Some of the cells of the sucker grow faster than others, and some, particularly at the sides, grow out in directions in which their neighbours do not follow. Some of these may penetrate more than one parenchyma-cell, growing straight through from side to side, dissolving a passage through walls and contents little if any larger than their own

diameter. One such cell is shown in Fig. 16, *a*, the red being the haustorial cell, the black the parenchymatous cells through which it has grown. Similar cells are shown in cross-section in Fig. 16 *b*, 16 *c*. These parenchymatous cells contain prominent nuclei, abundant protoplasm, and numerous chloroplastids. That one haustorial cell robs them of enough food to kill them is disproved by the fact that it may pass through three or four in its whole length, the contents of which are scarcely less than those of other and unattacked mesophyll-cells. There are no pathological appearances except the presence of the haustorial cell; both live, and the mesophyll-cell is apparently as well off as before it was attacked. The explanation is not far to seek. The mesophyll-cell makes and receives more food than it can consume. In a healthy leaf the excess of food made over what is consumed, is temporarily stored in its tissues, as the deposits of starch at certain times show, and this is later transferred to other parts of the plant. In those leaves which are penetrated by haustoria little if any starch is to be found. Why? Various causes perhaps combine to produce this result, but among them the haustorial cells must be considered important. The haustorial cells draw from the mesophyll in which they grow much or all of the starch formed in the process of assimilation which is not immediately consumed by the mesophyll-cells themselves. Unless too many haustorial cells attack the same mesophyll-cell it does not suffer sufficient loss to injure it to any apparent degree, but its value to the whole plant is greatly reduced or entirely destroyed by the intruder.

The cells of the sucker make their way in similar fashion through the parenchymatous tissues of the stem. Occasionally one finds a haustorial cell growing through cortical cells containing chlorophyll, just as in the mesophyll of a leaf, without causing any apparent harm to the cells, their protoplasm and nuclei seeming to thrive in spite of the intruder's presence. In the interfascicular and pith parenchyma, where of course no chlorophyll exists, such does not seem to be the

case, however. There the parenchyma-cells are killed and their contents entirely absorbed by the haustorial cell.

It is worthy of note that the papillate epidermal cells composing the cushion overlying the young haustorium, which make the first opening into the host, and the cells forming the sucker of the haustorium, have nuclei even larger than those of the majority of active cells in the parasite. Furthermore these nuclei are always situated near the tips of the cushion and sucker cells, where of course the activity of the cells is greatest.

Even after a complete union has been affected by the conducting tissues of the haustorium with one or two of the fibro-vascular bundles in the host, the cells which compose the sucker often continue to grow for some distance into the pith-parenchyma. Some of these which have penetrated deepest into the pith, having dissolved all the starch and other contents of the cells into which they have grown, seem at last to reach their limit of growth. Their tips enlarge until their walls become applied to the walls of the parenchyma-cells in which they have buried themselves. If these parenchyma-cells be large the effect is curious. A long sucking-cell, retaining a uniform diameter till it reaches the last cell into which it penetrates, becomes abruptly larger, blown out like a bladder against the walls which confine it. One cannot suppose that these enlarged tips are for the purpose of anchoring the haustorium firmly in the tissues of the host, for they are not formed until the haustorium has been for some time imbedded in the host, when the need of such anchoring, if there ever was any, has passed. They seem simply the final effort of the cells which form them to secure food in a comparatively innutritious pith.

A comparison of the growth of the haustorium of *C. americana* in the tissues of leaf and stem with what has just been described, shows that it penetrates its host in essentially the same way. In leaves where the mesophyll consists of large cells, one finds the cells of the sucker growing through mesophyll-cells which continue to live. The stems of the hosts examined contain little chlorophyll, and in them the haustorial cells kill those

into which they penetrate. In the pith one finds the ends of some of the longest sucking-cells enlarged, and their walls applied to the walls of the cells which enclose them.

The question as to the chemical nature of the solvent by which the papillate epidermal cells overlying the young haustorium dissolve the walls and contents of the epidermal cells of the host, and of the solvent by which the terminal cells of the haustorium perforate or entirely dissolve the deeper tissues of the host, still remains to be answered. I hope to throw some light on the matter in a subsequent paper.

CUSCUTA EPILINUM, Weihe, and CUSCUTA EPITHYMUM, Murr.

In 1880 Ludwig Koch published a long and careful paper[1] in which he describes these two species in all the stages of their life-history. He was unsuccessful, however, in finding such an elaborate structure in the haustorium, or such intimate histological and physiological relations between host and parasite as have just been described in *C. americana* and *C. glomerata*. I have, therefore, ventured to re-examine them.

The specimens of *C. epilinum* were grown on *Linum usitatissimum*, L. The stems of this parasite are considerably smaller and smoother than those of the two species which I have just described; they twine from left to right in generally long loose spirals, sending haustoria into the leaves as well as stems, but making altogether a looser investment of their foster-plants than does either of the foregoing forms. The haustoria are sometimes grouped along the stem, sometimes uniformly distributed. Their origin is deep in the cortex, as Koch has clearly shown. As Fig. 17 shows, they resemble in form, though considerably smaller, the haustoria of those plants with which we have already become familiar. They make their way into the host by openings effected in its epidermis through the solvent action of the thin-walled, papillate, epidermal cells which first come in contact with the host. They press through these openings, pushing aside the cortical

[1] L. Koch, Die Klee- und Flachs-seide. Heidelberg, 1880.

cells of the host which surround them, and carrying with them for a short distance the compressed cortical and epidermal cells which immediately overlaid them in the mother-plant. The sucking-cells at their tips, springing *all* of them from the shafts, penetrate the cells into contact with which they come by the same processes as previously described. I have been unable to find in the hosts, beyond the first few layers, any cells which come from the ordinary cortex of the parasite. The young haustorium does push some cortical cells into the host, but only for a very short distance. These are elongated by the pressure; they do not *grow* in as one might infer from their shape; they are *pushed* in, and do not long survive; they form part of the sheet of compacted cell-wall which enwraps the haustorium on its entrance into the host. We must return to the older idea of Mohl[1], cited by Koch, that these haustoria are roots only, not thalloid structures to which several tissues contribute, as Graf zu Solms-Laubach[2], with whom Koch agrees, is led to believe by his studies on other haustoria.

As already shown by L. Koch, a well-developed haustorium imbedded at its distal end in the tissues of a host consists of a central strand of tracheids, the rather thick walls of which are marked with large deep pits or coarse reticulations. I have seen no spiral or annular markings. Their absence seems to add some confirmation to the theory that they are formed only in cells which must be strengthened in a way which will not interfere with their elongation. Since the haustorium, like all roots, grows only at its tip, manifestly no such provision is necessary; and the greater lightness of the spiral or annular thickenings is not a desideratum since the structure does not have to maintain even its own weight, but is imbedded.

This strand of tracheids, united at the base of the haustorium by two lines of tracheids developed in the interfascicular parenchyma, with the xylem of generally two bundles in the

[1] H. v. Mohl, Ueber den Bau u. das Winden der Ranken u. Schlingpflanzen. Tübingen, 1827.

[2] Graf zu Solms-Laubach, Das Haustorium der Loranthaceen, Abh. d. nat. Ges. zu Halle, Bd. XIII, Heft 3.

mother-plant, is applied at its distal end against the ring of wood made by the confluent xylem of the host. The contact of haustorial xylem-cells with the xylem-cells of the host is therefore by their tangential walls, and is direct. When the haustorium has penetrated an oldish branch or stem this is the permanent condition; but if a younger part is attacked, one in which the xylem has attained to no great thickness, the cells at the apex of the haustorium remain thin-walled at their tips, continue their solvent action, and presently make their way through the zone of wood into the pith. No very wide opening is made through the wood, and comparatively few cells emerge into the pith. Such as do make their way into the pith are noticeably larger in diameter than those in the main body of the haustorium, are very much longer, and have thinner walls. They grow through the pith in all directions, some attacking the ring of wood in various places along its inner face, but never making much impression upon it; most cease to grow before they have reached the wood. Some of the smaller cells of the sucker which make their way into the pith grow only for a short distance, and their tips become enlarged as previously described. When the ring of wood has been thus penetrated, the central xylem-strand of the haustorium bifurcates at its tip and its tracheids apply themselves by their radial walls against the radial walls of the xylem-cells of the host, their thick and thin places corresponding with the thick and thin places of the ducts with which they come into contact.

So much has already been shown by Koch. Does the haustorium of *C. epilinum* consist merely of this central strand of xylem enclosed by elongated parenchyma-cells the conducting power of which is slight, or are there sieve-tubes as in *C. americana* and *C. glomerata*? Koch did not find any. In a cross-section of the host through a well-developed haustorium one finds on each side of the central xylem-strand one or two rows of cambiform cells, long and narrow (see Fig. 17, *c*). Beyond these are one or two rows of larger cells of equal or slightly greater length, resembling sieve-tubes

in appearance. Surrounding these are ordinary elongated parenchyma-cells the lumina of which contain abundant protoplasm. Those in the outermost row abut against the cortical tissues of the host. If such a section be treated as before described with an aqueous solution of aniline-blue, the cross-walls of some of these cells immediately outside the cambiform cells are coloured blue (see Fig. 17, *s*). The colour is not intense, for the walls are never much thickened, yet it is sufficient to prove the presence of callus. In older haustoria it is possible to demonstrate that these sieve-tubes unite with the sieve-tubes, their companion-cells with the companion-cells, of the host. One sees, too, that these haustorial sieve-tubes are connected, at the base of the haustorium, by strands of sieve-tubes developed in the inter-fascicular parenchyma, with the sieve-tubes of generally two bundles in the mother-plant.

The haustoria of *C. epilinum* agree, therefore, in structure with those of the two species already described. They consist of a central cylinder composed of a bi-collateral fibro-vascular bundle, two phloëms, with cambiform cells between, bounding a common xylem; and a cortex, thick at the base, reduced to a single layer of cells near the apex. The smaller size of these haustoria, and the correspondingly smaller size of their component cells, are the only differences between them and those previously described.

A similar investigation of *C. epithymum* gives similar results. The material at my disposal consisted of *Calluna vulgaris*, Salisb. over which *C. epithymum* had grown vigorously, sending its haustoria into leaves and stems. The haustoria were much broader in proportion to their length than in the other species, and the cells of the sucker were proportionally larger. Fig. 18 shows a young haustorium cut in a plane not parallel with its long axis, but at right angles to the long axis of the stem of the host. Owing to the plane of the section and the youth of the haustorium, its structure is not completely shown. One finds in this, however, that the shaft consists of a cortex and central cylinder; that

the central cylinder contains an axial strand of tracheids, a strand of cambiform cells bounding this on either side, and two phloëms. Staining with aniline-blue and partial decolorizing will demonstrate the presence of thin callus-plates on the cross-walls of certain of these phloëm-cells. They are therefore sieve-tubes. Other cells in the phloëm are shown by their proximity to the sieve-tubes, and by their abundant protoplasmic contents, to be companion-cells. A transverse section of the host through a sufficiently old haustorium will show that its xylem-cells connect directly with the ducts of the host, and are connected by xylem-strands with one or two axial bundles in the stem of the mother-plant. The xylem of the haustorium forms an unbroken connection between the xylems of parasite and host. In the same section, after treating with aniline-blue, the union of the haustorial sieve-tubes with those of the host, and the connection of the haustorial phloëm-groups with the phloëm of one or two axial bundles in the stem of the mother-plant, can be clearly seen.

A study of the origin and development of the haustoria of *C. epithymum* shows that, as Koch has said, they agree with those of *C. epilinum*. But as in *C. epilinum*, I am unable to find that the fully developed haustorium consists of any elements which do not come by division from the first two or three rows, deep in the cortex of the mother-plant, which give rise to the young conical haustorium. The haustorium as it grows, pushes the cortex and epidermis before it, finally ruptures them in the centre over its apex, pushes them still further by its continued growth, and carries them for a little distance into the body of the host. Then they quickly die. They have no use; they can neither absorb nor conduct readily; they in no way add to the efficiency of the haustorium, which, by its bi-collateral central bundle, is well able to transfer all the solutions which it draws from the conducting tissues of the host. The haustorium of *C. epithymum* corresponds in origin, structure, and function with the other haustoria which we

have studied in this paper. Its morphology and physiology are the same; it is a lateral root modified in structure to conform with the special conditions to which it is exposed and the special work which it has to do. It differs from the other haustoria only in its smaller size, and the consequent smaller size of its elements; it is structurally and physiologically as perfect.

CUSCUTA EUROPAEA, L.

The haustoria of this plant differ in no essential respect from those of the preceding species. They are larger than those of *C. epilinum* and *C. epithymum*. In them I have found the same bi-collateral structure of the central cylinder, a strand of tracheids bounded by cambiform cells which in turn are bounded by sieve-tubes and companion-cells. The conducting elements of both xylem and phloëms unite directly with the corresponding elements in the host, and furnish an unbroken connection between the conducting tissues of the parasite and its host.

One might have concluded from *a priori* considerations that such successful parasites as the Cuscutas must have their haustorial structures well adapted for the conduction of nutrient solutions of various sorts which they must draw from their hosts, and that these conducting tissues would be applied to the corresponding tissues of the host-plants in such ways as to do their work most perfectly. After the comparatively small amount of food stored in their seeds is consumed, the Cuscutas are absolutely dependent upon their hosts for food. Since they contain extremely little chlorophyll they must draw non-nitrogenous foods, sugar, and so forth. For the further elaboration of these they must have certain mineral salts and inorganic nitrogen-compounds. We should therefore expect to find, what has long been known to be the case, that the haustoria contain xylem-elements for the conduction of these last two sorts of substances. It is through the sieve-tubes that carbohydrates are conducted. We should therefore expect to find

sieve-tubes in the haustoria. This is now shown to be the case. Through the haustoria these parasites are supplied with all the food-substances which they need.

It may be asked if all parasitic Phanerogams possess both sieve-tubes and tracheids or tracheae in their haustoria. It is evident from the structure of the haustoria of the species of *Cuscuta* studied, that these plants can absorb, not only those substances which are conducted through the xylem-elements of their hosts, but also those conveyed by phloëm-elements. We know that, at times at least, especially in spring, the reserve food-materials of our perennials, converted into their soluble forms in the places where they are stored, are conveyed through the ducts to the points where they are needed. At these times, the ducts contain sugars and other organic substances, besides water and the crude substances absorbed by the roots. It might have been supposed that, owing to the attachment of haustoria, the host of a *Cuscuta* was forced to use its stored food at once, after converting it into soluble form and conveying it through the ducts to the places of consumption, and that the *Cuscuta* drew away, through the tracheids which were long ago found in its haustoria, only, or little more than, these reserve matters. The presence of sieve-tubes in the haustoria, and their direct union with the sieve-tubes of the host, prove that the parasite can and does abstract the recently elaborated food substances in its host, as well as the reserve matters.

We can now put the question in a more definite form. Do all phanerogamic parasites possess the appliances for abstracting from their hosts both reserve food-substances and also those freshly formed ; or are some forced, by the absence of haustorial sieve-tubes which unite with the sieve-tubes of their hosts, to depend on reserve matters only? In order to be able to answer this query in part at least, I have examined the haustoria of *Viscum album*, L., two members of the Rafflesiaceae, *Brugmansia Zippelii*, Bl. and *Rafflesia Patma*, Bl., and also *Balanophora elongata*, Bl.

The material of *Viscum album*, preserved in strong alcohol,

was collected in 1891 in the Botanic Garden at Bonn, and consisted of mature fruits, fruits from which the roots were just penetrating the cortex of the host, young two-leaved seedlings, and older stages of one and two years growth. The host was *Crataegus monogyna*, Jacq.

The root from a germinating seed makes its way by solution and pressure through the cortex of its host into and through the phloëm, and penetrates for a little distance into the young wood. The form of the haustorium thus imbedded is wedge-shaped, rather than conical. A tangential section of the host (a transverse section of the haustorium) shows that the young haustorium consists of two sharply defined regions, a cortex composed of rounded cells, rich in protoplasm, and with large spherical nuclei; and a central region, elliptical in form, made up of more or less rectangular cells, those in the centre being thick-walled. A cross-section of the host (a radial section of the haustorium) shows that the cortex of the haustorium is composed of nearly isodiametric parenchyma-cells with small intercellular spaces, that the cells at the periphery are thicker walled than those farther toward the centre, and that they abut against the somewhat compressed cells of the cortex and bast of the host; that the central cylinder consists of tracheids in its centre, and of cambiform cells with large spindle-shaped nuclei surrounding these. No sieve-tubes are visible even when the tracheids have become numerous and thick-walled.

In older plants, the haustorium sends through the cortex, parallel to and near the surface of the host, branches radiating in various directions and almost encircling the stem. A careful study of the main trunk of the haustorium (which in its mode of penetrating the host resembles a tap-root), and of its branches, shows only one structure that is not found in the younger stage. The tracheids are more numerous and unite directly, not at the tip, but at the sides of the haustorium, with the xylem of the host. Bordering the tracheids are the cambiform cells, with abundant protoplasmic contents and spindle-shaped nuclei; but absolutely no sieve-tubes are

to be found. The haustorial cells near the phloëm of the host are strong and thick-walled, and they abut against compressed, often dead, cells. The strand of tracheids, although these unite directly with the xylem-elements of the host, is not connected with the fibro-vascular bundles of the mother plant. The cambial ring of the host, severed by the young intruding haustorium, is joined again through the formation of a cambium in the haustorium. This cambium, the one new structure above referred to, cuts the strand of tracheids. Across this stratum of cambium, consisting of from two to several layers of cells with no intercellular spaces between them, the liquids drawn from the wood of the host, and conducted for a short distance by the haustorial tracheids, must be transferred. On the side of the cambium toward the periphery of the stem, the tracheids are by no means as regularly arranged as on the inner side, and the body of the borer may be massively developed, while as yet no vessels have been fully formed at its point of origin in the cortical root [1].

We thus see that in the haustorium of *Viscum album* provision is made for the conduction into the parasite of those substances in the xylem only of the host, and that this conducting system is by no means mechanically perfect. Being incomplete in structure, its physiological efficiency must also be imperfect.

The alcohol-material of *Brugmansia Zippelii*, *Rafflesia Patma*, and *Balanophora elongata*, very generously put at my disposal by Professor A. F. W. Schimper, was collected by him in Java in 1890, and was in excellent condition. Every stage in the development of these plants was represented, except the seeds, either ripe or germinating.

For the question in hand, it was necessary to examine sections of tolerably advanced stages only, in order to determine the presence or absence of sieve-tubes; but for the sake of completeness, I have repeated some of the work of earlier writers, and have studied other stages.

[1] De Bary, Comp. Anat. of Phanerogams and Ferns, p. 384. Clarendon Press, 1884.

If one makes a transverse section of a root of *Cissus sp?* about two or three centimeters in diameter, which is attacked by *Brugmansia Zippelii*, one finds in the cambium, and in both phloëm and xylem, cells which are clearly distinguishable from the ordinary cells of these tissues by the very large spheroidal nuclei which they contain (see Fig. I, *a*, Pl. XIV, and Fig. X, *a*, Pl. XV). Those in the cambium are smaller and have thinner walls than those in the phloëm and xylem. These latter are also more or less deep brown in colour. In the xylem, one finds the cells with large nuclei between those elements which have only very recently been developed from the cambium, or between those in which starch is commonly stored. Seldom are they in contact with the ducts. Those near the cambium may be attached to one another; they are also rather thin-walled. Those deeper in the xylem are generally isolated and thick-walled, especially if they are in contact with cells in which there is no starch. In the younger phloëm, the cells with large nuclei are in rows of two or three and are thin-walled; but in the older phloëm they are usually isolated and thick-walled, with larger or smaller vacuoles. It sometimes happens that one finds a row of six or seven of these cells extending from the cambium to the older parts of the phloëm, and in such cases (see Fig. I, *a*, Pl. XIV) the progressive thickening of the walls from those in the cambium outward is very noticeable. In the cambium, and in both xylem and phloëm, these cells are found between the ordinary elements of these three tissues, and they exercise no pressure sufficient to compress their neighbours. They seem so much like normal elements of these tissues that they are readily distinguished from them only by their large nuclei, and the brownish hue of their contents.

In a radial section through a bundle of the same root, one finds that in the cambium these cells with large nuclei are arranged in chains (see Fig. III, Pl. XIV, and Fig. IV, Pl. XV, cells in red). That these chains, which do not always run for any great distance parallel to the plane of the section, are continuous, is demonstrated by serial sections. They branch

frequently. The branches grow either in the cambium-layer, or to the wood and bast. In the wood and bast (see Fig. IV, Pl. XV, red cells) the chains become broken and the cells isolated. The reason for this is simple. The cells which compose the chains found in the cambium, by their continued growth and division lengthen the chains. The cells which compose the branches running to the wood and bast do not long continue to grow, or to divide. Their neighbours do grow, however, and these branches, being in radial lines to and from the centre of the stem, are pulled apart by this growth. The process of isolation takes place most rapidly in the phloëm, for reasons which will be given presently. These cells grow and multiply in the cambium; they grow only slowly, and do not multiply, in the wood and bast.

If now a cross-section of a *Cissus*-root be made through one of the larger, more or less hemispherical swellings that are found at irregular intervals on its surface, one sees a structure similar to that shown in red in Fig. VI, Pl. XV. An examination shows that it is composed of a great number of cells, the majority of which are exactly like those just described. These swellings are the more or less advanced stages in the development of the floral structures of *Brugmansia*. As already shown by Graf zu Solms-Laubach[1], they are formed by the multiplication, at certain points, of the cells which compose the chains above described. This is clearly shown in Fig. I, Pl. XIV, where part of a young bud is connected with a branch of one of these chains penetrating the phloëm of the adjacent bundle. The formation of a floral structure begins in the interfascicular cambium adjacent to a bundle, as shown in Fig. V, Pl. XV. The multiplication of the cells which compose the chain takes place at first more rapidly on the side toward the centre of the stem, forming a more or less conical structure composed entirely of thin-walled parenchyma-cells, rich in protoplasm, rather dark brown in colour. This structure is rather closely applied to

[1] Die Entwickelung der Blüthe bei *Brugmansia Zippelii*. Botanische Zeitung, 1876.

the fibro-vascular bundle by the side of which it grows, and it pushes its apex deeper and deeper into the medullary ray, the cells of which contain much starch.

In the same way, but less rapidly, the structure grows toward the surface of the root, pushing up the overlying cortical tissues. One side is applied to the phloëm of the adjacent bundle. It is noticeable that the growth of this structure causes very few cells of the host to collapse. This is accounted for by the fact that, at first, it does little more than keep pace with the rapid growth of the whole root. Presently, however, that part of the now rather spindle-shaped mass of cells which lies beyond the cambium toward the periphery, begins to grow more rapidly. Thus the overlying tissues are compressed and pushed upward, forming the increasingly large hemispherical swelling on the surface of the root (see Fig. VI, Pl. XV). As already shown by Graf Solms [1], the growth of the parenchymatous mass now becomes very slow, except at the outer end of the spindle. A definite growing-point is formed, from the sides of which the bracts and floral envelopes are successively cut off (see Fig. VI), while from its apex the stamens and pistil are later developed.

Just before the first bracts appear, the differentiation of the first fibro-vascular bundles takes place, in a region several cell-layers from the sides of the now top-shaped mass of parenchyma, and about in a line with the older parts of the phloëm of the adjacent bundles of the host. These young bundles originate, and remain, distinct from one another, their development taking place both upward as the bud grows, and downward toward the base of the bud. The bundles are very simple, collateral, open, composed of short tracheids and xylem-parenchyma, of several layers of cambium-cells, and of short phloëm-elements. As the bundles develop, a direct union is effected between the tracheids and the adjacent xylem of the host, and the phloëm unites, in similar fashion, with the phloëm of the host.

Only in buds well advanced in development is it possible to

[1] loc. cit.

distinguish the different elements composing the phloëm of the bundles; yet in a thin section, stained with an aqueous solution of aniline-blue, the presence of callus-plates in the narrow sieve-tubes is made evident (see Fig. VII, Plate XV, red lines represent callus-plates). The sieve-tubes of these bundles unite directly with the sieve-tubes in the adjacent bundles of the host, as is shown in Fig. VIII (the brown shading represents cells of the host, the red of the parasite). A conducting system is thus installed such as we have already found in the haustoria of the Cuscutas. A study of the further development of the buds is beyond the scope of the present paper.

Owing to the lack of germinating seeds it is impossible absolutely to determine the morphology of what has been so far described; but, from analogy with *Viscum album* for example, we may infer that the seed, germinating on the surface of the host-plant, sends a primary root into its tissues, and that this root soon branches. This root and its first branches may be composed of differentiated tissues. In *Viscum*, as shown above, the branches grow in the cortex near the surface of the host. In *Brugmansia*, the primary root of the seedling probably does not branch so near the surface, but rather in the cambium of its host. Such at least seems to be the case in *Pilostyles Hausknechtii*, Boiss., as described by Graf Solms[1]. These branches, making their way through the cambium, have no need of conducting tissues, their cells being individually nourished, like those of the cambium, from the adjacent tissues. They are therefore composed only of thin-walled parenchymatous cells. In the root of *Cissus* we recognize the branches as the chains of cells already described. By the rapid multiplication of their cells at certain points buds are formed, beginning as small masses of parenchyma, which later increase in size, and in which fibro-vascular tissues are differentiated. The chains of cells growing in the cambium have, as their sole function, to nourish themselves, a very easy matter owing to their position.

[1] Ueber den Thallus von *Pilostyles Hausknechtii*. Botanische Zeitung, 1874.

When they branch into the wood or bast of the host, they are not in so favourable positions for securing food, they are not so well nourished, their cells cease to grow and presently become separated from one another. But those branches which penetrate into the wood find starch stored in parenchymatous cells, and can therefore survive for a long time; whereas those which grow into the bast have no such stores to draw upon, and sooner become inactive and dead, after thickening their walls. An extreme case of thickening is shown in Fig. II, Plate XIV.

The chains of cells in the cambiums are probably, therefore, extremely reduced roots composed entirely of merismatic cells. Professor Strasburger has suggested the name of *embryonic tissue*, since from this, as from the simple tissue of the embryo, the new plant is developed. Graf Solms [1] has compared these chains to the mycelium of a fungus—'Die Vegetationsorgane der Rafflesiaceae sind auf einen gliederungslosen, intramatricalen Thallus reducirt, der häufig völlig den Charakter eines Pilzmyceliums annehmen kann'—but the evidently cellular character of these chains makes the comparison, though suggestive, misleading. It seems to me better to call these chains of cells growing in the cambium, morphologically roots, but so reduced in structure that they are nothing more than embryonic tissue.

In *Rafflesia Patma*, Bl., as Graf Solms [2] has clearly shown, there are similar chains of parenchymatous cells, growing in precisely the same positions in the host (see Figs. IX and X, Plate XV), and giving rise to buds which eventually develop into flowers. In these buds and flowers we find conducting tissues of two sorts, tracheids and sieve-tubes, which are directly united with the corresponding tissues of the host.

In these two parasites the provisions for securing and conducting to a considerable and, until the seeds are ripe,

[1] Engler and Prantl—Die natürlichen Pflanzenfamilien, 35 Lieferung, 1889; Rafflesiaceae.

[2] loc. cit. p. 275.

a constantly increasing distance from the host, an abundant and varied food supply, is as complete as in the Cuscutas.

Treatment of thin sections of fairly developed buds of *Balanophora elongata*, with an aqueous solution of aniline-blue, shows that the fibro-vascular bundles contain sieve-tubes, and that both the sieve-tubes and the tracheids are united directly with the corresponding elements of the host.

We see, then, that in parasites belonging to three distinct families (Convolvulaceae, Rafflesiaceae, Balanophoreae), sieve-tubes are developed in the haustoria. But no sieve-tubes were found in the haustoria of *Viscum album*. The parasites in the haustoria of which sieve-tubes have been found are absolutely dependent upon their hosts for food, they have no green tissues in which food can be elaborated. *Viscum album*, on the contrary, is abundantly supplied with chlorophyll, both in leaves and stem. It demands only that its host shall supply it with aqueous solutions of the crude materials from which it can elaborate its own food. It is a 'water-parasite'; its host performs for it only the functions of a root, absorption, conduction, mechanical support.

Is it possible that other green parasites are thus only partially dependent, and that they too, drawing from their hosts only unelaborated substances, form in their haustoria only tracheids or tracheae, and no sieve-tubes? I hope to pursue the question further.

I wish to express my grateful appreciation of the encouragement which Professors Strasburger and Schimper, and Dr. Schenck, by their kind criticism and suggestions, and the generous supply of material, have given me.

BONN AM RHEIN, *February*, 1893.

EXPLANATION OF FIGURES IN PLATES XIII, XIV, AND XV.

Illustrating Mr. Peirce's paper on the Haustoria of Phanerogamic Parasites.

PLATE XIII.

Cuscuta americana, L.

Fig. 1. Parasite twining about twig of *Mimosa*, *a*; *b*, *Mimosa*-twig showing perforations by haustoria; *c*, parasite nat. size, and *d*, enlarged, showing haustorial discs.

Fig. 2. Cross-section of young *Cuscuta*-stem 2-3 cm. above youngest haustorium. ×20.

Fig. 2′. Cross-section through abortive haustorium. ×20.

Fig. 3. Radial section through vascular bundle of *Cuscuta*-stem. *E*, toward epidermis; *p*, parenchyma; *a*, air-cavity; *d*, pitted ducts; *c*, cambium; *cc*, companion-cell of sieve-tube *s*. ×125.

Fig. 4. Radial section of *Cuscuta*-stem showing young haustorium imbedded in cortex and overlaid by 'cushion'; haustorium-cells only in outline. ×75.

Fig. 4′. Development of 'cushion-cells' from epidermal. Nuclei large and near tips of cells. ×220.

Fig. 5. Cross-section of stem, showing young haustorium. *e*, epidermis; *g*, growing-point. ×125.

Fig. 6. Cross section of parasite (red) and host (brown); bifurcation of haustorial xylem at *b*. *Mimosa*. ×20.

Fig. 7. Cross-section of host (longitudinal of haustorium); host brown, parasite red shading, red lines are callus-plates; *s*, sieve-tubes; *t*, tracheids; *t′*, union of *t* with xylem *x* of host; *c*, cambium. ×75.

Fig. 8. Cross-section of parasite (red) and host (brown); bifurcation of haustorium at *f*; *b*, fibro-vascular bundles of host; *c*, cortex; *p*, pith. *Apocynum sp?* ×20.

Fig. 9. Cross-section of parasite (red) and host (brown); *P*, *Cuscuta*; *k*, cork of host; *c*, cortex; *H*, haustorium; *S*, sclerenchyma; *sb*, soft bast; *w*, wood; *p*, pith.

Fig. 10. Cross-section of haustorium in mother-plant: *e*, epidermis; *c*, cortical parenchyma; *d*, cortex of haustorium; *g*, central cylinder with young bundles *a*, *b*. ×20.

Fig. 11. Cross-section of haustorium, bundles *a* and *b* of Fig. 10; *p*, phloëm; *c*, cambium; *t*, tracheids. ×220.

Fig. 12. Longitudinal section through haustorium to show union of its xylem and phloëms with two bundles of mother-plant; *t*, young tracheids; *s*, *s*, young sieve-tubes; *H*, basal region of haustorium; *C*, interfascicular parenchyma; *E*, direction of epidermis and tip of haustorium; *l*, latex-tubes. ×220.

PLATE XIV.

Fig. 13. Cross-section of haustorium (tangential section of host) showing union of haustorial sieve-tubes, *a* with large sieve-tubes *S* of host: *H*, haustorium; *S*, sieve-tubes of host; *c*, callus-plates between host and haustorial sieve-tubes. × 500.

Cuscuta glomerata, Choisy.

Fig. 14. Longitudinal section of haustorium in *Impatiens Balsamina*, showing union of haustorial tracheids and sieve-tubes with ducts and sieve-tubes of host; callus-plates in red; *t*, union of tracheids and tracheae by tangential walls. × 220.

Fig. 15. Tip of haustorium penetrating mesophyll of leaf; haustorial cells red, host plain. × 220.

Fig. 16. Cells of tip of haustorium growing through mesophyll-cells: *a*, in optical longitudinal section; *b*, *c*, in actual cross-section; haustorial cells red; *n*, nucleus; *v*, vacuole; *p*, chloroplastids of mesophyll-cell. × 500.

Cuscuta epilinum, Weihe.

Fig. 17. Longitudinal section of haustorium in *Linum usitatissimum*, showing union of haustorial tracheids and sieve-tubes with ducts and sieve-tubes of host: host brown, parasite red, callus-plates as red lines; *c*, cambium; *s*, sieve-tubes of haustorium: *h*, hard bast; *p*, soft bast; *m*, cambium; *w*, wood of host. × 125.

Cuscuta epithymum, L.

Fig. 18. Longitudinal section of haustorium in *Calluna vulgaris*; young and not parallel with long axis of haustorium, hence junction of haustorial and host tissues only partly shown; *s*, sieve-tubes with (red) callus-plates. × 120.

Brugmansia Zippelii, Bl.

Fig. I. Cells of *Brugmansia* (in red) in phloëm and medullary ray of *Cissus sp.*? root (in brown); *s*, sieve-tubes of *Cissus*; *a*, chain of *Brugmansia*-cells in contact with young bud, *b*. × 125.

Fig. II. Much thickened cells of parasite (red) in old phloëm of host (brown). × 220.

Fig. III. Longitudinal section of *Cissus*-root showing chain of *Brugmansia*-cells growing in cambium: parasite red, host brown; *x*, xylem; *c*, cambium; *s*, sieve-tube. × 125.

PLATE XV.

Fig. IV. Longitudinal section of *Cissus*-root with *Brugmansia*-cells going to xylem and phloëm: parasite red, host brown; *x*, xylem; *c*, cambium; *s*, sieve-tube with *c'*, companion-cell. × 125.

Fig. V. Cross-section of *Cissus*-root showing young and quite undifferentiated floral structure of *Brugmansia* in medullary ray and adjacent to a bundle; *p*, phloëm; *c*, cambium; *x*, xylem; *ifc*, interfascicular cambium; *mr*, medullary ray of host (brown). × 125.

Fig. VI. Half-developed bud of *Brugmansia* in section, showing a swelling on surface of *Cissus*-root. × 2.

Fig. VII. Longitudinal section of vascular bundle of *Brugmansia*; callus-plates of sieve-tubes in red. × 125.

Fig. VIII. Direct union of sieve-tubes of *Brugmansia* (red) with sieve-tubes of *Cissus* (brown); callus-plates as red lines. × 125.

Rafflesia Patma, Bl.

Fig. IX. Chain of cells of parasite (red) growing through cambium of root of host (brown) and branching to xylem and phloëm; *x*, xylem; *c*, cambium; *p*, phloëm. × 125.

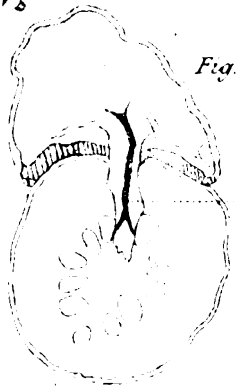
Fig. X. Cross-section of host-root showing mode of growth and appearance of parasite-cells (red); *x*, *c*, *p*, as before. × 125.

Fig. 1.

Fig. 2.

Fig. 2'

Fig. 6.



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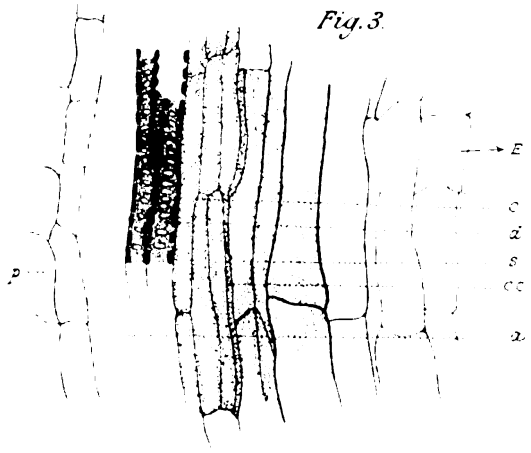

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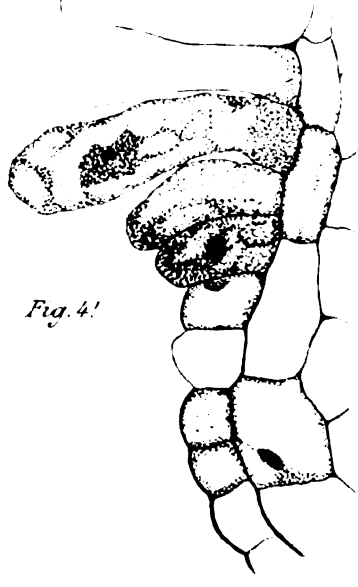

Fig. 8.

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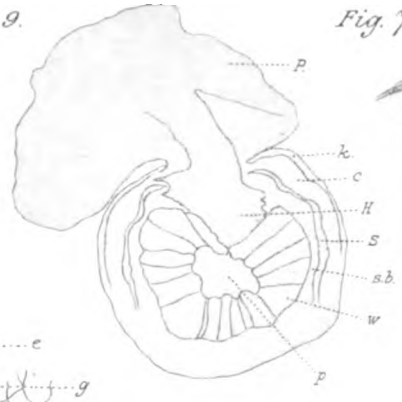

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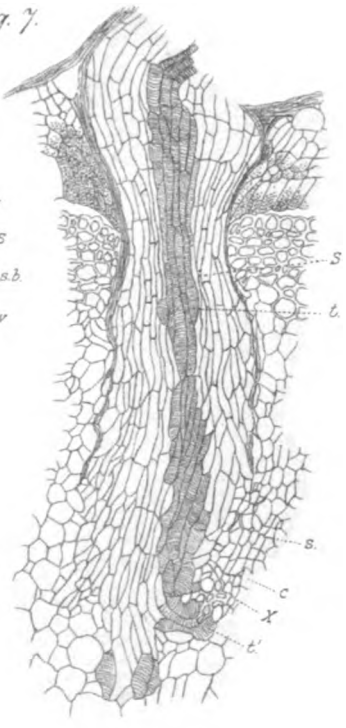

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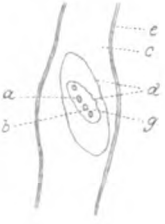

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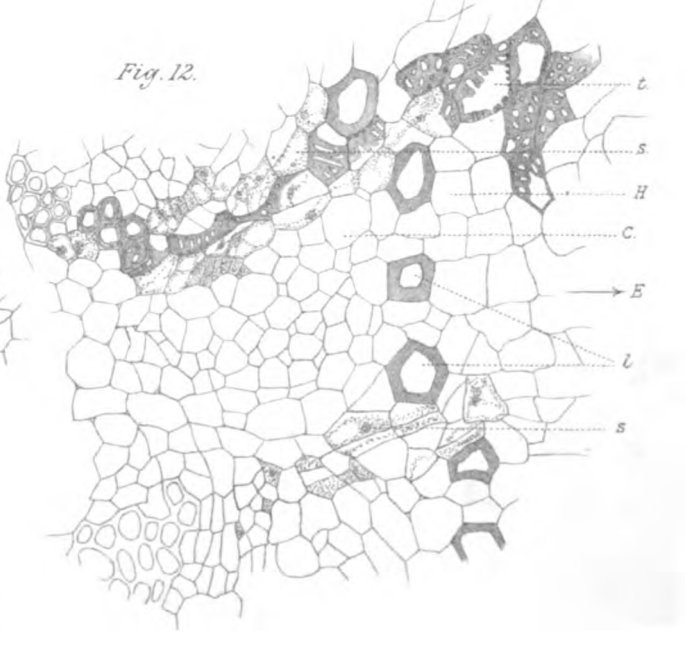

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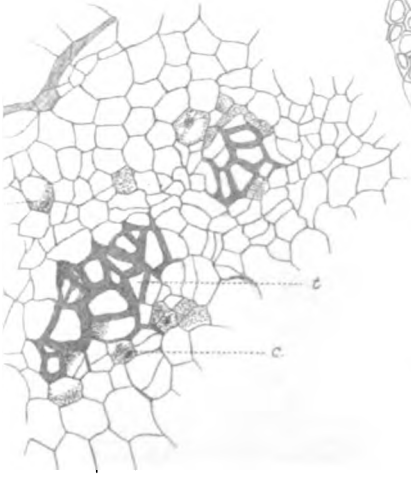

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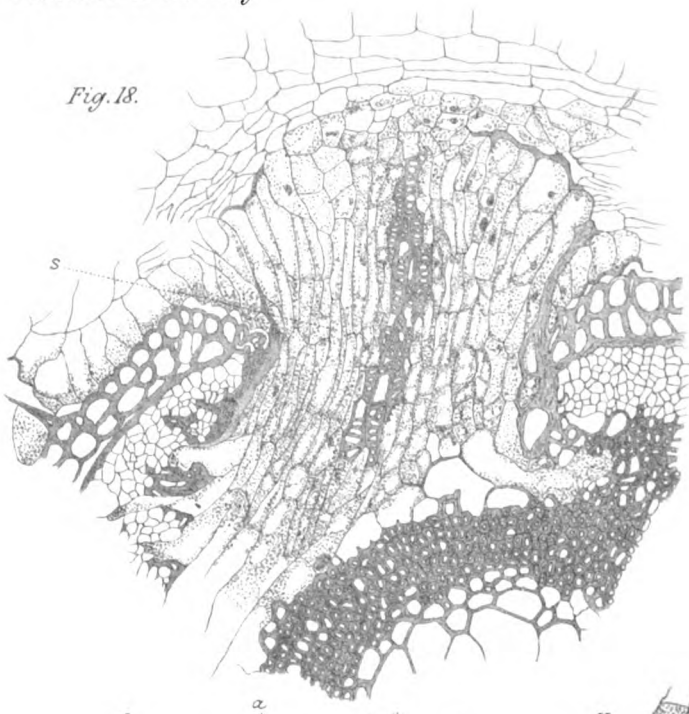

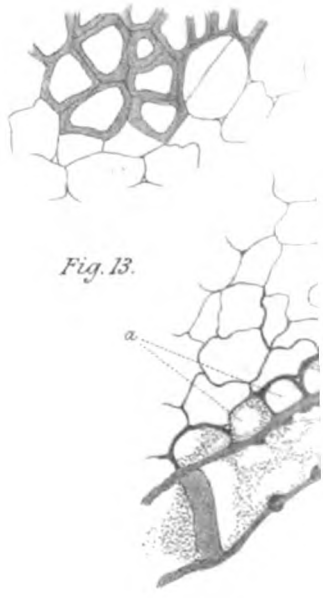

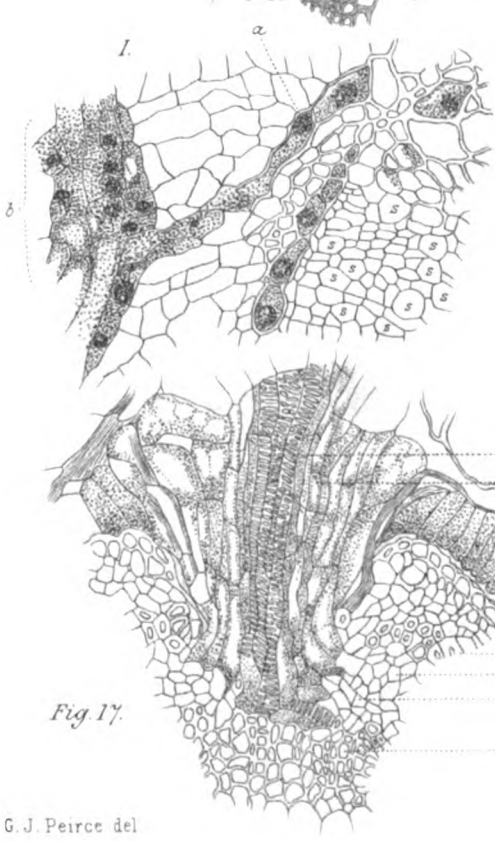

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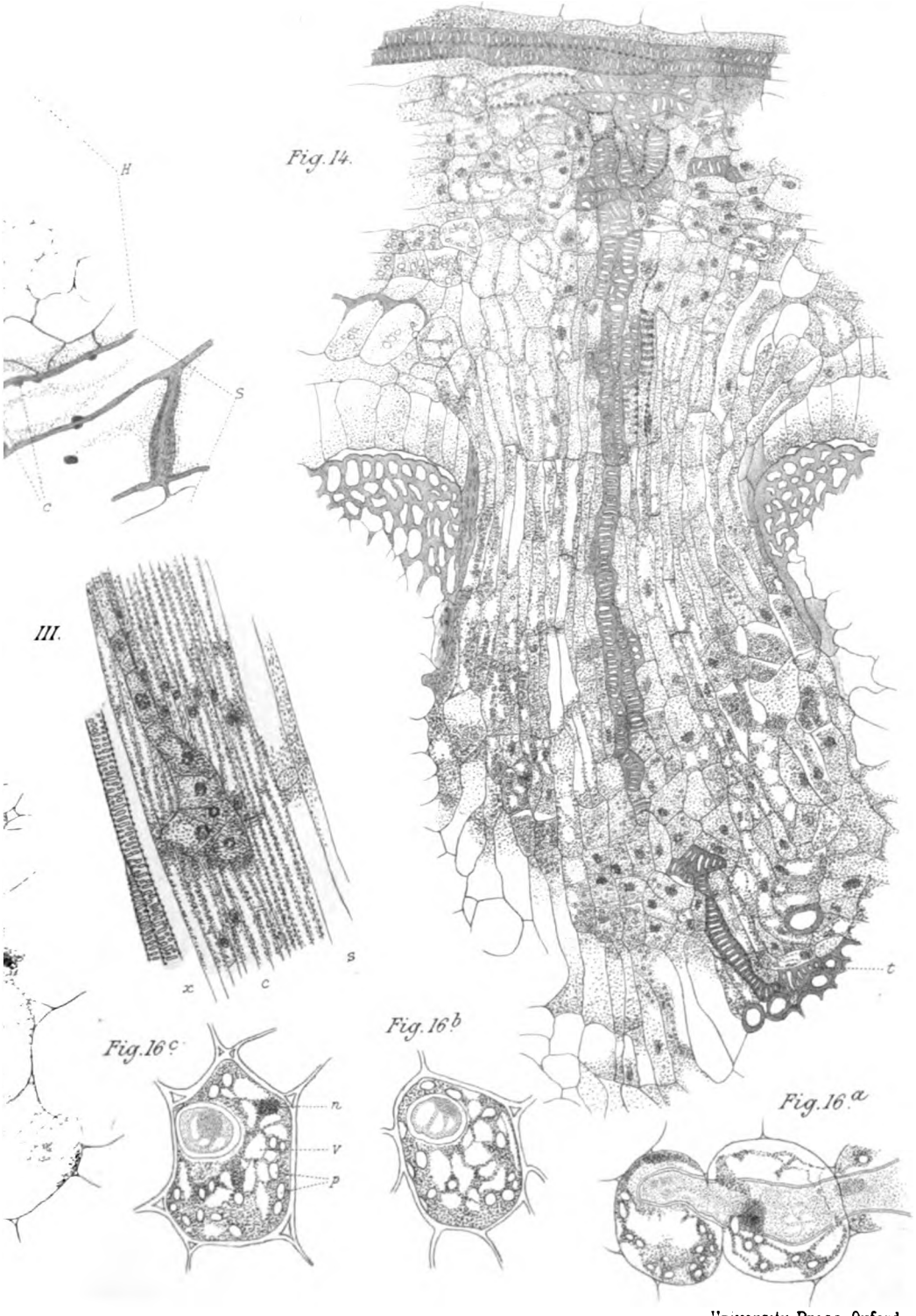

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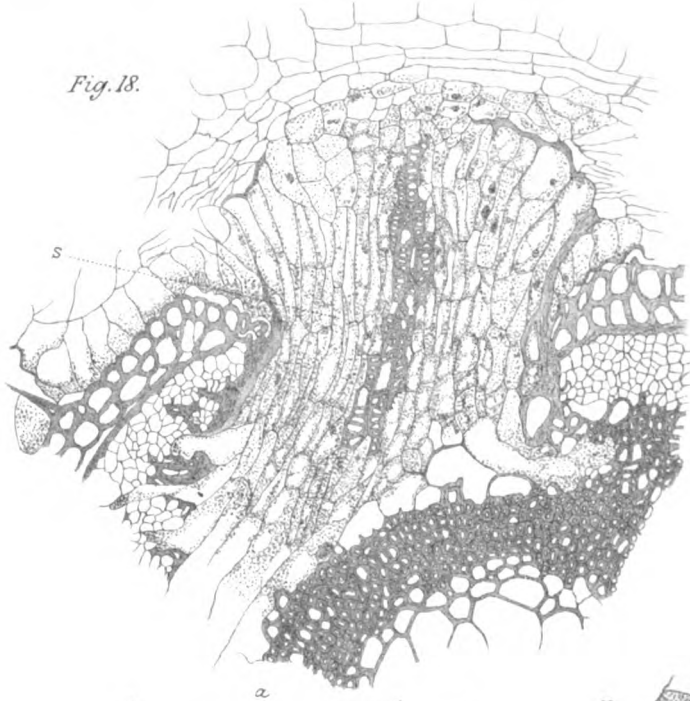

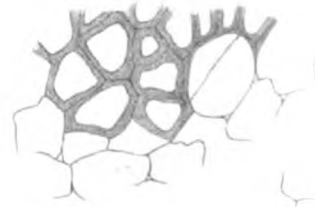

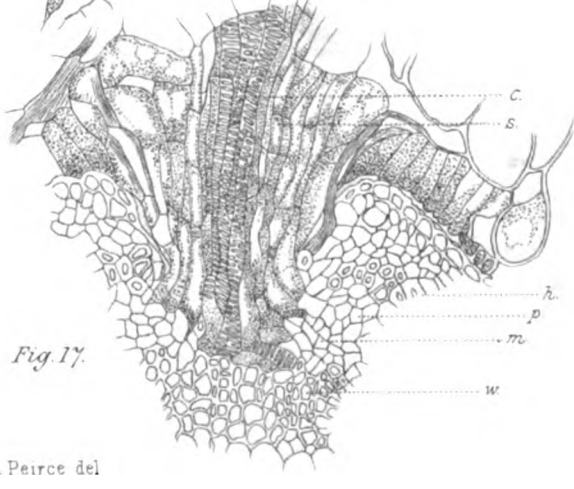

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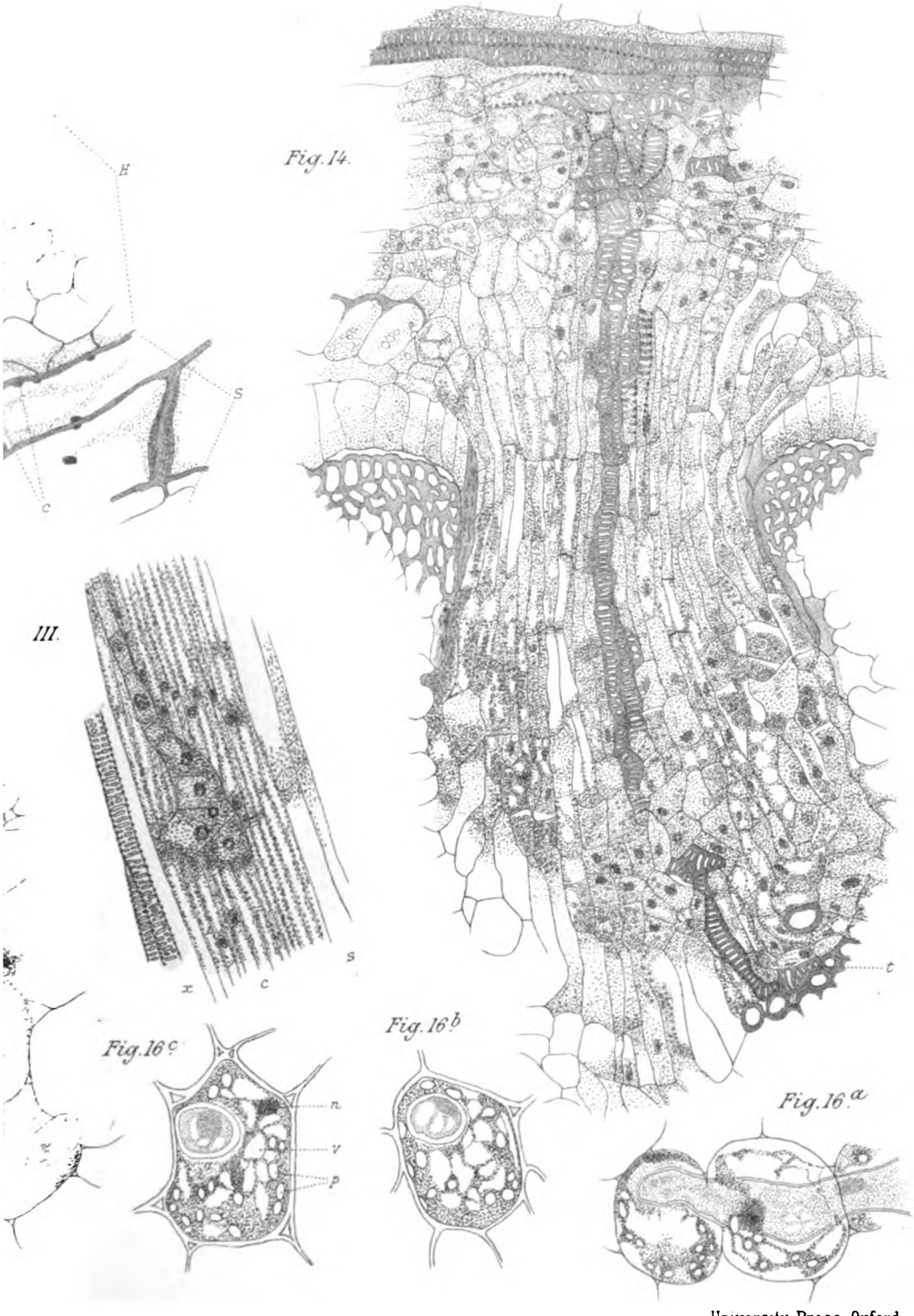

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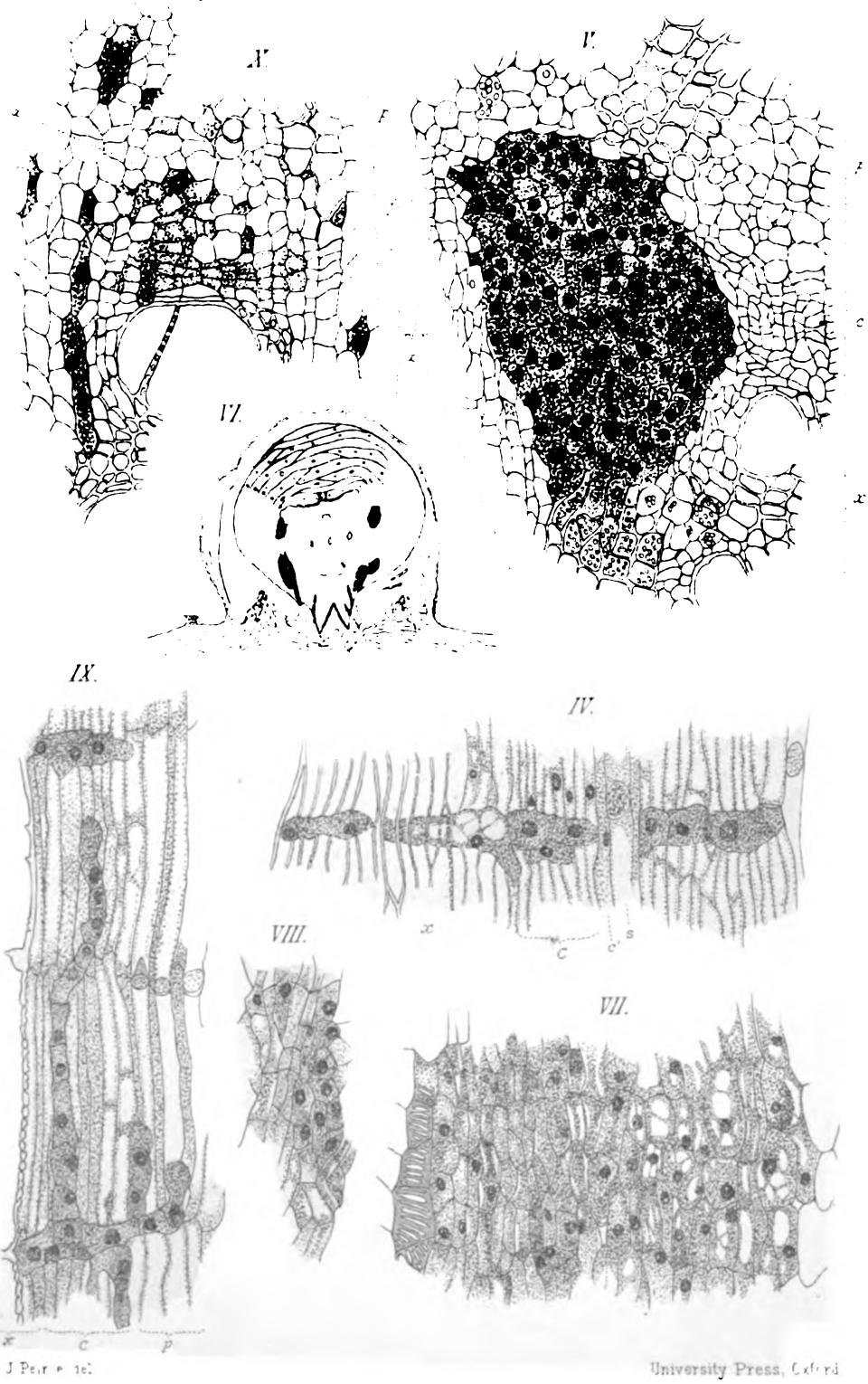

J. J. Pearce del. University Press, Oxford

PEIRCE — PHANEROGAMIC PARASITES.

On the Structure of the Axis of Lepidostrobus Brownii, Schpr.

BY

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With Plates XVI and XVII.

THOSE who examine sections of fossil plants microscopically are only too familiar with the gaps which occur in their tissues: these may have been due to the existence of lacunae in the living plant, or they may owe their origin to imperfect preservation after death of tissues present in the living state; or both of these factors may combine to produce that discontinuity of tissues which is so frequently found in such specimens. It is only in the most perfectly preserved fossils that it is possible to decide how far the gaps present are due to the one or the other of these causes, while developemental evidence, which in the anatomy of modern plants would be at once called in to solve such questions, is usually unattainable in fossils.

In some cases there can be little question that the lacunae were present in the living plants: this conclusion may be based upon examination of the tissues and comparison of

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living forms; as an example *Astromyelon Williamsonis* may be taken[1]. Here the cells bordering on the lacunae are so well preserved, and the whole nature of the tissue so similar to what is commonly found in living aquatic plants which show schizogenetic spaces, that the conclusion is justified that the lacunae existed in the living state, with essentially similar characters to those now seen in the fossil.

As a second example *Rhizonium lacunosum* may be cited[2]. Here the cortex has clearly been lacunar, as in *Astromyelon*, but many of the trabeculae of tissue are broken, and the question arises whether they were broken during life or subsequently; it seems not improbable from the appearance of the section represented in the figure which has been quoted, that the rupture was made after death.

It will hardly be necessary to remark that the most difficult cases for decision will be those where the cavities are lysigenetic; to draw the line between the natural destruction of cells and tissues in the normal life of the plant, and the disorganization of the softer tissues after death, but before complete fossilization, will clearly demand the most careful examination of well-preserved specimens.

It is well known that large gaps are commonly present in the tissues of *Lepidodendron*, and they are found, not only in the normal stems and Stigmarian roots, but also in the axis of the strobilus.

In transverse sections of Stigmarian rootlets, two spaces are commonly found: an outer large space in the cortex, in which the central vascular strand is suspended, being sometimes attached by a bridge of tissue to the peripheral band of cortex[3], and an inner space immediately surrounding the strand of xylem. Let us consider first the outer of these spaces, viz. that in the cortex. Professor Williamson gives

[1] Organization of Fossil Plants of the Coal Measures. Williamson, Phil. Trans., Pt. II, 1883, Pls. 27–30.

[2] Williamson, Phil. Trans., vol. 180 (1889, B. Pl. III, Fig. 23).

[3] Compare Williamson, Monograph on *Stigmaria ficoides* (Palaeontographical Society, 1886). Pl. XIII, Fig. 79.

a figure[1] of a rootlet cut transversely near its base, in which the outer space is filled with a continuous tissue of thin-walled cells; this is, however, the case only at the extreme base; rather higher up a large cavity is found within the peripheral band of cortex, and surrounding the central vascular strand, and this apparently extends throughout the length of the root. At the extreme base the 'rootlet cushion,' as Williamson styles the continuous tissue at the base of the rootlet, is found to be covered by minute branching tubular cells[2], which project upwards and outwards into the large cavity, while some form oblique connecting filaments which run to the outer band of cortex. Williamson further remarks[3] that these cells are 'frequently much disorganized,' while they are seen only at the base of attachment of the rootlet to the main root. Taking these facts into consideration, together with the details of development of the roots of *Isoëtes*, in which a somewhat similar space occurs[4], and also the fact that in the internal roots of *Lycopodium Selago* the outer cortex may be found completely separated by an air-space of schizogenetic origin from the inner cortex, it will seem probable that the outer space in the Stigmarian rootlet was chiefly of schizogenetic origin, though filamentous cells such as those above quoted appear also to have been present, at least in early conditions of the rootlet, and to have undergone disorganization, either during life or prior to complete fossilization; their disorganization would thus contribute lysigenetically to the formation of the cavity.

Turning now to the inner space of the Stigmarian rootlet, though this is commonly vacant, it is sometimes found to be partially or completely filled with a tissue to which the character of phloëm is ascribed[5]. It seems probable that in the living state this inner cavity did not exist, but that it

[1] loc. cit. Pl. IX, Fig. 51.
[2] loc. cit. Pl. X, Figs. 43 and 50.
[3] loc. cit. p. 27.
[4] See Naegeli and Leitgeb., Beitr. zur wiss. Bot., Heft IV, p. 132, and Pl. XIX.
[5] See Williamson, loc. cit. p. 32, Pl. XI, Fig. 62.

represents the space which was occupied by the delicate tissue of the phloëm.

Similarly, cavities are found in the stems of the various species of *Lepidodendron*. The bulky cortex in these is usually distinguishable into three bands, of which the middle band is rarely preserved; in some species there is still very little detailed information as to the nature of this tissue; and even in most of those cases where it has been recognized as a continuous tissue the state of preservation does not appear to have been sufficiently good to allow of detailed description[1]. Suggestions have been made in certain cases that this middle layer consisted of spongy parenchyma, and so forth; but till very recently little was positively known of the parts which are so frequently missing. The tissues immediately outside the xylem, presumably of the nature of phloëm and protective sheaths, together with the innermost band of cortex, are also commonly absent[2], or imperfectly preserved[3]. Quite recently, however, M. Hovelacque has published a most exhaustive description of the structure of the stem of *Lepidodendron selaginoides*, Sternb.[4] He has studied the entire succession of tissues which compose the stem; he finds the tissues of this species, when well preserved, to be a continuous sequence, without discontinuity or large lacunae, and distinguishes three chief zones of the tissues outside the central vascular stele; of these his inner cortex (écorce interne) presents the most interesting characters for comparison with the results to be described below. It is composed of (1) the protective sheath, which is simply the innermost layer of cells; (2) the inner zone, consisting of thick-walled cells; (3) the middle zone, often destroyed, consisting of thin-walled cells with intercellular spaces; (4) the outer zone of thick-walled cells. The zones (2), (3) and (4) appear to correspond essentially to

[1] Compare Solms' figure of *L. selaginoides*, Will., Fossil Botany, Fig. 23, p. 221. Engl. ed.: also Figures by Williamson, Phil. Trans. Part II, 1881, &c.

[2] Renault, Cours de Botanique fossile, vol. ii, Pl. 4, Fig. 3, and Pl. 10, Figs. 4, 5.

[3] Williamson, loc. cit., Pl. 51, Fig. 10, &c.

[4] Mém. d. l. Soc. Linn. de Normandie, vol. xxii, Fasc. 1.

those zones of somewhat similar nature which may be distinguished in the cortex of some living species of *Lycopodium*, or those above noted in other *Lepidodendra*. M. Hovelacque has also traced with great detail the progress of the leaf-trace from the central stele to the leaf-cushion, its structure, and relations to the surrounding tissues, and his results are interesting for comparison with those to be described below.

Notwithstanding that in certain cases, such as the specimens of *Lepidodendron selaginoides* thus described by M. Hovelacque, the tissues appear as a continuous sequence, the occurrence of large gaps in many specimens calls for further consideration, while a comparison with living plants of *Lycopodium* and *Selaginella* will be important for the elucidation of the results. The discontinuity which is common in vegetative stems of *Lepidodendron* comes out even more strongly in most specimens of the axis of *Lepidostrobus*; in these the tissues are often badly preserved, especially the phloëm and the middle cortex. A brilliant exception is, however, found in the large cone of *Lepidostrobus Brownii* in the British Museum, known there as Brown's cone. This silicified fossil shows, in an unusually perfect state of preservation, both the tissues of the phloëm and of the inner and middle cortex. These will be described in detail below, in the hope that the knowledge of them may assist in the investigation of the vegetative axes; it is not, however, assumed that the details to be described would necessarily apply for other species, as there is probably a considerable variety of structural minutiae in the different forms, while it is even possible that the details of the fertile and sterile axes might differ in the same species.

The fossil is already well known by the description given of it by Robert Brown[1], and it is extraordinary that investigators, having his description before them and the sections readily accessible, have not subjected them to fresh examination in the light of the more recent advances in Palaeophytology. Graf Solms, referring to this fossil, quotes Schimper's

[1] Linn. Trans. XX, p. 469, &c.

figures of it, which do it very scant justice[1]; and as regards the central axis he merely remarks that, 'The axial strand, which is surrounded by numerous transverse sections of leaf-traces, has the structure of *Lepidodendron Harcourtii*'[2]. On a previous page Solms has pointed out (p. 226) that this name covers two distinct species, and of these Brown's cone seems to resemble the form styled by him *L. Williamsoni*, as regards the structure of the axis. The distinctive points are (1) the absence of secondary thickening; (2) the central parenchymatous pith; (3) the sinuous outer limit of the xylem, the point of insertion of the leaf-trace bundles appearing in the transverse sections as sharp, outward-pointing teeth; (4) the absence of bast-fibres from the bundles of the leaf-trace. All these characters, which are illustrated in the drawings of *L. Brownii* to be described below, correspond to those given by Solms for *Lepidodendron Williamsoni*.

Turning now to the details which may be observed in the sections preserved in the British Museum, it must first be stated that I confine the present observations to the structure of the axis; a detailed description of the sporangia is being prepared for publication elsewhere.

Fig. 1 is a copy of a photograph which represents the axis cut transversely, together with the bases of thirteen sporophylls. At the centre is the bulky parenchymatous pith, which merges, without any sharp limit, into the narrow dark-looking ring of xylem; this has a crenulated margin, and gives off from the projecting points bundles of the leaf-trace, which at first traverse a dense inner band of ground-tissue. As they reach the margin of this band, the tissue directly surrounding them becomes more lax and filamentous, till they finally emerge into the wide clear space intervening between the central mass of tissue and the dark peripheral band of the cortex. In this space the leaf-trace bundles appear as isolated dots; but in their oblique course they ultimately reach the peripheral band of cortex, traverse it, and finally pass out-

[1] Solms, loc. cit. Figs. 25 A, B, p. 233.
[2] Fossil Botany, p. 238.

wards to the sporophylls. This course of the bundles is further illustrated by the longitudinal section (Fig. 2), in which they may be seen taking their oblique course upward through the clear intervening space from the solid central mass into the dark peripheral band of the cortex. Special attention should be paid to the oblique bundles at the apical end of the section; here a felt of fine filaments connects the bundles, while still in the intervening space, with the neighbouring tissues. These filaments have already been figured by R. Brown[1], but they appear to have entirely escaped the notice of all recent writers on the subject. It will be one of the objects before us to examine the nature and origin of these filaments.

Having now taken a general view of the axis in transverse and longitudinal section, we may proceed to the more detailed examination of the tissues under higher powers (Fig. 3 A). Starting from the centre, the bulky pith (*p*) (characteristic of *L. Williamsoni*) shows no features calling for remark; its large thin-walled cells pass over gradually into the thick-walled tracheides of the wood (*xy*), and remind one of the relations of the undeveloped, as distinct from the fully matured xylem of some concentric bundle. The figure shows well how irregular is the outer limit of the woody mass, the summits of the crenulations of the peripheral part being occupied by smaller tracheides, presumably including the protoxylem. The middle of the part shown in the figure is occupied by one of those projecting teeth, from which a leaf-trace is just about to be given off. The tissues outside the wood are in a remarkably fine state of preservation, considering how delicate they must have been: a point to note especially is the presence of a layer, which I believe to be an *endodermis* (*sh*); it is recognized by the more sharp definition of its walls, while in parts the radial walls still appear to show the characteristic dot. Again, on following the sheath in its sinuous course round the central vascular mass, the radial walls of the sheath are found ruptured, in exactly the way so often to

[1] Linn. Trans. XX, Pl. XXIV, Fig. A.

be seen in sections traversing the endodermis of living plants, owing to the brittleness of the walls of that tissue. The above characters point to the conclusion that the sheath in question is an endodermis, though it is to be noted that it is not always so recognizable as in the part of the section selected for our Fig. 3 A. Clearly this endodermis will correspond in position to the innermost layer of the cortex, distinguished by M. Hovelacque as the 'Gaine'[1]. He did not, however, recognize any marking on the radial walls of the cells[2]. The tissue lying outside this endodermis is the inner dense band of the cortex, consisting of parenchyma, with sclerenchymatous elements, which may be scattered singly, or, as the outer parts are reached, they may preponderate, and form a dense mass of tissue (Fig. 7), in which the bundles of the leaf-trace are embedded. Returning to the tissue which lies between the ring of wood and the endodermis, the characters appear to be ill-defined—the band of tissue is of variable thickness, being sometimes reduced to but two layers (Fig. 3 A, opposite the projecting tooth), or it may increase to four or more. I have not been able to recognize any characters distinctive of soft bast beyond the delicate cell-walls, and in any case, if a true phloëm was present, it can have existed only in comparatively small quantity. There is, however, nothing to preclude the idea that this tissue represented the phloëm, such as it was.

A comparison of this central stele with that in other Lycopodinae shows that it is most nearly matched by that in the Psilotaceae. With most species of *Selaginella* the correspondence is not close: apart from the peculiar character of the endodermis in that genus, the vascular arrangement is quite distinct in most species; in the larger species, however, such as *S. Willdenovii*, where three large vascular bundles are seen in the transverse section, these show at times a crenulated margin: but the nearest resemblance is naturally to be expected in the sub-genus *Selaginella* proper, with multifarious leaves, such as *Selaginella spinosa*, P. B.;

[1] loc. cit. Figs. 11, 12 (*g*, *g*).
[2] loc. cit. p. 148.

but even there, apart from the peculiar development of the endodermis, the correspondence is not so close in details of the cylindrical stele as that in the Psilotaceae proves to be. The absence of any definite layer of cells of the endodermis in *Lycopodium* is a distinctive feature; and though in some species (e.g. *L. Selago*) the xylem may be united into a connected stellate body, as seen in transverse section, still the correspondence is not in any way a close one. On comparing with the Psilotaceae, however, the following points of resemblance may be noted—(1) the central thin-walled parenchyma resembles that well known to occur in *Tmesipteris*, though its place is taken in *Psilotum* by thick-walled sclerenchyma; moreover it is to be remembered that this thin-walled central tissue is not a constant character in *Lepidodendron*: (2) the crenulated margin of the xylem corresponding to the star-like xylem of the Psilotaceae: (3) the definite layer of endodermis, corresponding to that already noted by De Bary[1] in *Psilotum*: this is shown in Fig. 3 B, which should be carefully compared with Fig. 3 A drawn from Brown's cone, when the detailed resemblance of the two cannot be missed. M. Bertrand[2] describes the endodermis in *Psilotum* as not definitely characterized, and difficult to distinguish, and this is borne out by his Figs. 173–176, though in his Figs. 161, 162, he draws the endodermis with the characteristic marking. I find, however, that though the cells of the endodermis do not form a perfectly regular layer, there is no difficulty in tracing the sheath, after suitable treatment, by the usual characteristic structure of the radial walls. (4) The very slight bulk of the tissue referable to the phloëm, and the absence of distinctive characters in it. De Bary noted the narrowness of the band in *Psilotum*, and a similarity of some of the elements to the sieve-tubes of Ferns; this I can confirm. The closeness of correspondence of the tissues in the section of *Lepidostrobus* (Fig. 3 A) to that of *Psilotum* (Fig. 3 B)

[1] Comp. Anat., Engl. ed., p. 348.

[2] Arch. Bot. d. Nord. de la France, 1, p. 401.

is certainly very striking, both as regards arrangement and structure, allowance being made for the much smaller size of the plant of *Psilotum*. The correspondence with *Tmesipteris* is not so close; M. Bertrand distinguishes under the name 'gaîne protectrice' a somewhat irregular sheath surrounding the vascular stele [1], after examining this sheath in various stages of development. I do not find the characteristic marking of the radial walls, nor is the sheath itself in any way clearly defined as regards arrangement of cells. In fact *Tmesipteris* appears in this respect to correspond to *Lycopodium*; on the other hand, *Psilotum* and *Lepidostrobus Brownii*, while they resemble one another in the details of the sheath, differ from *Tmesipteris* and *Lycopodium*; the comparison with *Selaginella* as regards the sheath will be introduced later. Turning to the point of the presence or absence of hard woody tissue in the centre of the cylinder, the closer correspondence is between *Lep. Brownii* and *Tmesipteris*, since in both the central tissue is thin-walled, while in *Psilotum* it is sclerenchymatous; thus the histological characters noted in the plants above cited furnish us with a most interesting example of cross-correspondence.

Finally, as regards the presence of a stellate, connected central xylem, there is a certain resemblance between our *Lepidostrobus* and, not only the Psilotaceae as above noted, but also with certain species of *Lycopodium* (*L. Selago*) and of *Selaginella* (*S. spinosa*), and such examples serve to draw together the Lycopodinae on anatomical grounds, notwithstanding that in many species of these genera divergent arrangements appear to predominate.

As it has already been shown elsewhere [2] that there are points of similarity between the sporangia of *Lepidodendron* and the sporangia of *Tmesipteris*, the close correspondence in respect of details of internal anatomy of the stem between *Lepidostrobus Brownii* and the Psilotaceae, as above demonstrated, becomes more specially interesting.

[1] loc. cit. Figs. 210–216.

[2] Proc. Roy. Soc., vols. 53 and 54; meetings of Feb. 16 and June 15, 1893.

It has been already stated that the bundles of the leaf-trace are given off from the projecting teeth of the central stele, such as that shown in the middle of Fig. 3 *A*.

Our next study will be to follow such a bundle on its course outwards, and consider its structure and its relations to the surrounding tissues. The general course pursued by the leaf-trace in this cone is shown in Fig. 2 to be obliquely upwards from the point of origin. The band of firm ground-tissue which surrounds the central stele is first traversed; here the bundle shows such structure as is seen in the transverse section in Fig. 4—it is of considerable bulk (though this appears to be variable, Fig. 4 *B*) and consists of a well-defined mass of xylem (*xy*) directed towards the centre of the axis, and of less clearly defined tissue, probably phloëm, which is on the peripheral side (*ph*. Fig. 4 *A*); the whole is surrounded by an ill-defined sheath of thin-walled cells, in which the characters of endodermis or pericycle cannot be recognized with any degree of certainty. It is to be specially noted that many of the cells of this sheath have divided so as to duplicate the sheath (cells marked *x*, Figs. 4 *A* and 4 *B*), a point which may frequently be observed round similar bundles of *Lycopodium* and *Tmesipteris*. It has been found impossible to recognize the protoxylem with certainty: in the wood of this bundle (Fig. 4) there is no clear indication of it, either by size or marking of the tracheides; nor does examination of the longitudinal sections (Fig. 9) materially help, beyond the fact that the tracheides on the central limit have a slightly closer thickening than those lying further outwards; accordingly it is impossible to make a definite statement as to the number of initial points of development of the wood; it may, however, be remarked that such a bundle as Fig. 4 *A*, *B*, which is most excellently preserved, gives no support to the view that the bundle is diarch, as has been stated as a distinctive character for *Lepidodendron*, though (as I think) on slender evidence, by Renault[1]; still less does it appear that the bundle is of

[1] Cours de Bot. Foss., III, p. 11.

the concentric type. The question has been discussed by Solms[1] as to the type of bundle of the leaf-trace in *Lepidodendron*, and has been left open, owing to the insufficiency of evidence: in the case of *L. Williamsoni*, to which species our fossil appears to correspond in its general characters, the evidence is in favour of the collateral type[2]. The remarkably well-preserved bundle shown in our Fig. 4 *A* seems to me to show clearly, as regards *L. Brownii*, that the bundle of the leaf-trace was of the collateral type; it is to be noted, however, that this character does not come out so clearly in all the leaf-trace bundles of this cone (compare Fig. 4 *B*).

The results of M. Hovelacque as regards the structure of the leaf-trace bundles in *Lep. selaginoides* are interesting for comparison[3]: he finds the bundles to be of the collateral type, more distinctly so than in *Lep. Harcourtii*; the size of the bundle varies on its course outwards, being largest in the middle cortex. He describes the bundle as being surrounded by a sheath, but this is not clearly defined. In these broad characters there is a general correspondence with the results above described for *Lepidostrobus Brownii*, but the variations of structure illustrated in Figs. 4, *A* and *B*, make it difficult to draw detailed comparison.

Of a number of species of *Lycopodium* which have been examined for purposes of comparison, *L. Phlegmaria*, one of the large-leaved species, showed the nearest correspondence of structure of the leaf-trace of our *Lepidostrobus*; a section is shown in Fig. 4 *C*; the very small proportion of tissue referable to the phloëm is a remarkable feature, while the number of cells which have divided by walls parallel to the limit of the bundle (marked *x*) is to be noted for purposes of comparison with the *Lepidostrobus*.

A comparison of transverse sections of the leaf-trace of *Tmesipteris* shows correspondence of structure quite as close as this; as examples, the Figs. 217, 218, 231 of M. Bertrand[4]

[1] Fossil Botany, Engl. ed., pp. 219, 226. [2] loc. cit. p. 226.
[3] loc. cit. Figs. 20–37 and pp. 150–152.
[4] Arch. Bot. d. Nord de la France, Vol. i, p. 500, &c.

may be quoted, though it must at the same time be admitted that the similarity depends rather upon the absence than the presence of definite characters in the tissues.

Such being the structure of the bundle in its course through the inner cortex, it must now be traced outwards to the more lax tissue of the middle cortex, and the first change which appears is the elongation of such cells as are marked (x) in Fig. 4 *A* and *B*; intercellular spaces also appear between them (Fig. 5), and the bundle thus becomes surrounded by a lax, lacunar tissue (Fig. 6). The enlargement of the spaces and elongation of the cells finally results in the partial (Fig. 7), or complete isolation of the bundle (Fig. 8, *A* and *B*), from the surrounding ground-tissue, except in so far as a connexion is maintained by the elongated trabeculae. It will be at once seen that these trabeculae, originating as they do from the irregular sheath surrounding the bundle, are very similar to the trabeculae of *Selaginella*. This comparison is strengthened by the apparent occasional presence of a ring-like zone round the trabeculae (Figs. 7 and 11), though this is not constantly to be seen, nor can much stress be laid upon such a point in dealing with a fossil. As the bundle approaches the outer limit of the inner dense zone of cortex, the trabeculae elongate and the intercellular space extends all round the bundle, so that it finally emerges into the large ring-like cavity, so clearly seen in Fig. 1. The bundles are here quite isolated in the fossil, and appear as dots in Fig. 1; as they approach the outer dense band of cortex, trabeculae extends inwards from it towards the bundle (Fig. 8 *A*), showing broken ends, while the superficial cells of the bundle show similar evidence of broken attachment. It would thus seem probable that the connexion of the bundles by means of the trabeculae to the cortical tissue was, at least during earlier life, more complete than is now seen in the fossil state.

The structure of the bundle as it traverses the space (Fig. 8) adds to the evidence of its collateral character; it is surrounded, as is commonly the case, in *Selaginella* by

a parenchymatous sheath of two or more layers in thickness; within this on the peripheral side (i. e. on the side nearer the arrow in Fig. 8 *A*) is a dark line, which is the probable limit between the sheath and the phloëm; the latter thus would consist of some two rows of elements on the side of the bundle nearer the periphery of the axis. The xylem in the bundles shown in Figs. 8, *A* and *B*, is rather irregular, and there is no clearly defined protoxylem.

A longitudinal section through such a bundle, as it traverses the large air-space, shows such structure as is seen in Fig. 9. At the periphery are still to be seen the points (*x*) where trabeculae have broken away; the tracheides show a closer reticulation on the central than on the peripheral side, but there is no more marked sign than this of a protoxylem.

Finally, the Figs. 10, 11, show, as seen under a lower power, the structure which is found towards the apex of the cone, when cut in longitudinal section (compare Fig. 2); in this region the intercellular space is narrower, and the bundles more crowded, so that the section here shows some approach to the state of an earlier stage of development. It is easily seen that the connexion of the bundles to the other tissues by means of the trabeculae is here more complete, while numerous trabeculae are seen to be broken away; such details are again shown under a higher power in Fig. 11, while some of the trabeculae appear to be marked by the transverse zone above alluded to. Certain of these points have been already illustrated by Sowerby in the plates attached to R. Brown's memoir[1], but have not been noticed by more recent writers.

After traversing the large air-space, the bundles enter the outer dense band of the cortex and finally pass out into the sporophylls, but their further behaviour calls for no special notice.

The details of structure above described show a remarkably perfect state of preservation, much better, in fact, than that

[1] Linn. Trans. vol. xx; see especially Pl. XXIV, Fig. A.

usually found in the calcified nodules. The facts acquired may assist in the interpretation of the less perfect lime-incrusted fossils, and will be of value for comparison with (1) other *Lepidostrobi*, (2) vegetative stems of *Lepidodendron*, and (3) living Lycopodinous plants. When the comparison is made with a fine transverse section of a *Lepidostrobus* from a lime-nodule, supplied to me by Mr. Lomax, the plan of structure of the axis is found to be virtually the same; the chief difference lies in the existence of vacant spaces, where the softer tissues of the phloëm and the trabecular cortex occur in Brown's cone. It seems probable that in this, as also in other cases of such cones, observed or figured by other writers, the complete structure was essentially similar to that above described. A point for remark is, however, the wholesale disappearance of the more delicate tissue; but if the limits of those tissues, which have been preserved in specimens from the lime-nodules, be carefully examined, it will be found that many of the cell-walls end abruptly, just as would be the case if, in such tissues as those shown in our Fig. 4, the darker lignified walls were preserved, while the more delicate, probably cellulose, walls were entirely broken down.

The structure of the vegetative stems of various types of *Lepidodendron* is well known, so there will be no need to enter on a comparison of the vascular tissues; our object will rather be to consider the nature of their cortex, and to compare it with that of Brown's cone. It will presently be shown that there is considerable variety in the mode of development of the cortex in the species of living Lycopods. The same appears to have been the case with the fossils. Three distinct bands of the primary cortex seem to be commonly present in the vegetative stem of *Lepidodendron*, exclusive of the tissues resulting from secondary change: they do not correspond to the three cylinders described by Solms[1], and illustrated by his Fig. 23, for these include

[1] Fossil Botany, p. 220.

also secondary tissues; what we have before us is the primary condition of the cortex, for purposes of comparison with living forms in which, as in *Lepidostrobus Brownii*, no secondary changes appear. In Solms' Fig. 23 of *Lepidodendron selaginoides* the dark zone of firm tissue, outside the vascular cylinder, but internal to the lax tissue which he describes as the 'inner cylinder,' corresponds to what I have described as the *inner cortex*. A similar firm band of cortical tissue surrounds the vascular stele in certain species of living Lycopods. The lax tissue, which in *Lepidostrobus Brownii* I have called the *middle cortex*, corresponds to the imperfectly preserved tissue, which Solms styles the 'inner cylinder,' while the dense and broad *outer cortex* of *Lep. Brownii* corresponds to the inner part of that broad band which Solms termed the middle rind, together with what remains of primary cortex outside the zone of thickening. It is unfortunate that this difference of terms should exist; it is due to the fact that while I have kept in view the primary condition of the cortex, and its differentiation in living Lycopods, Solms has based his terms upon fossils which show the results of secondary change. That the latter is unsatisfactory seems to me sufficiently plain from the fact that the 'middle rind' of Solms includes both primary and secondary tissues, while no account is taken by him of the innermost firm band of cortex. But, whatever be the terminology, the three bands of *Lep. Brownii* find their counterparts in the cortex of *Lep. selaginoides*; the correspondence is more evident on comparison of the larger and more detailed drawing of Williamson[1], and from this, other points of similarity may be traced, which are necessarily omitted in Solms' smaller-scale drawing. The inner firm band of cortex is also shown by Williamson in longitudinal section[2].

I have examined several specimens of *Lep. selaginoides* in my possession, and find the firmer band surrounding the

[1] Phil. Trans. 1881, Pl. 48, Fig. 4.
[2] Phil. Trans. 1878, Pl. 22, Fig. 34, g. g.

stele constantly present, though it may be only comparatively narrow. The leaf-trace bundles of this species show an interesting relation to that middle delicate band of cortex which is so often found more or less disorganized—it was, in fact, a tissue of a very spongy character, with large intercellular spaces, which were traversed by trabeculae consisting of one or more cell-rows. This is clearly shown in Fig. 12, which represents one of the leaf-trace bundles traversing the middle cortex of a lateral branch. The bundle (*vb*), which is badly preserved, was surrounded by a firmer band of cortex of some three or four layers thickness; but further from the bundle the spongy tissue is readily seen, consisting of very irregular trabeculae with large intercellular spaces. It is not to be wondered at that such a tissue should frequently be very ill-preserved, or even disappear altogether.

Other species also show a firmer band of cortex, internal to the soft middle band: thus in sections of the Laggan Bay *Lepidodendron* it is present, though only as a narrow band. In a section of a young twig of *Lep. Spenceri* also the three bands are seen clearly, while the middle band has a filamentous or trabecular character. But though those three zones of the cortex may be distinguished in some stems of *Lepidodendron* it must not be assumed that they are constant in all; thus in *Lep. Harcourtii*, as represented by Williamson[1], either the innermost firm band has never been present, or it has been disorganized; the latter is hardly probable where more delicate structures have been, at least in part, preserved. Though the greater part of the cortex up to the central stele was probably of a delicate texture in *L. Harcourtii*, as also in the above cases, there is no evidence of a trabecular structure such as that described for *Lepidostrobus Brownii*; my own specimen of *Lep. Harcourtii*, though imperfectly preserved, supports Prof. Williamson's observations. In other species (*Lep. fuliginosum*), in which the preservation is very perfect, the inner

[1] Phil. Trans. 1893, B. Pl. I, Figs. 1, 2, 3.

firm layer is not clearly differentiated, though the central stele is surrounded by a tissue which is slightly more dense than that which occupies the middle of the cortex; here a very peculiar condition of the tissue is found, for it consists chiefly of multicellular filaments which are intertwined together in irregular fashion (Fig. 13); the tissue resembles in its general character that of the central strand of *Fucus* rather than the tissue of a vascular plant. There is no special trabecular development of the tissue round the leaf-trace bundles as they traverse the cortex in *Lep. fuliginosum*.

From the above it will be gathered that there is some variety of detail in the characters of the cortex in the strobilus and vegetative axes of *Lepidodendron*, the differences chiefly depending upon (1) the relative thickening of the walls, and (2) upon the size of the intercellular spaces, and the position in which they appear relatively to the vascular system. There is, however, in most cases a differentiation of the cortex into three more or less distinct bands, of which the middle band was of a softer and often of a very spongy or even trabecular character.

In connexion with the structure of the cortex in various species and specimens of *Lepidodendron*, a comparative examination of the cortex in living species of *Lycopodium* and *Selaginella* is important. It is well known that there are considerable differences in the character of the cortex in various species of these genera, and it will now be pointed out that these are in many respects similar to those found in the fossils. In some species of *Lycopodium* the cortex is not clearly differentiated into distinct bands; this is the case in *L. annotinum*, where it is almost uniformly dense and thick-walled; on the other hand, in *L. carinatum*, *L. Hippuris*, and *L. Phlegmaria* the cortex is almost uniform in texture throughout, but the tissue is thin-walled, and permeated by intercellular spaces of small size. The same is the case in *L. dichotomum*, at least while young, though in old stems, where the roots pursue their internal course downwards through the cortex, large mucilaginous cavities are apt to

appear in the middle cortex. Such mucilaginous cavities are, however, a marked feature in the cortex of *L. inundatum*, while large intercellular spaces are found in the middle cortex of that species, and are traversed by plates of cells or trabeculae (Fig. 14); but beyond this the cortex of *L. inundatum* is not clearly differentiated. In the more common British species, however, the cortex is differentiated into distinct bands. *L. clavatum* shows a very dense and hard inner cortex, and a similar, though often narrower, dense band at the periphery, while between there is a lax thin-walled tissue, which is very readily broken down; the size of the intercellular spaces in this tissue is variable. Such characters are found both in the creeping vegetative stem and in the axes which bear the strobilus. In *L. alpinum*, in both vegetative and strobiloid axes, the inner cortex appears as a dense band; it merges gradually into a lax lacunar tissue, in which the cells are elongated into filamentous trabeculae very irregularly disposed. This spongy mass extends to the peripheral epidermis, so that the outer firm band of cortex is entirely unrepresented in this species (Fig. 15); this spongy tissue is very similar to the mesophyll of the leaves of the same plant, and the leaf-cushions may thus be described as extending over the surface of the axis.

In *L. Selago* there is a narrow band of inner cortex, not clearly marked, surrounding the stele, a broad band of thinner walled and more lax middle cortex, and an outer firmer band; but none of these are clearly defined, nor are the cell-walls lignified excepting the outer band, and that only at a comparatively late stage. The chief interest in the cortex of this plant lies in the middle band; for there, in old stems, the tissue shows that peculiar appearance of interlaced filaments which has been alluded to as existing in *Lep. fuliginosum* (Fig. 13). In *L. Selago* the peculiarity is shown in a less degree, but it is quite similar in kind. Lastly, in *L. nummularifolium*, Blume, the cortex shows the converse of what is seen in *L. alpinum*, the peripheral part being dense, with thickened walls, while the inner tissues are relatively thin-

walled. Certain species of *Selaginella* correspond to this last type, in respect of the structure of the cortex. *S. Martensii* and *S. Willdenovii* show a peripheral, firm and woody band, which becomes more delicate on passing inwards, till finally the lax trabeculae round the bundle are reached. *S. spinosa* is also of the same type, but in this species the sclerosis is limited to the epidermis [1], while the inner part of the cortex is not only thin-walled, but contains large lacunae, which open into the air-space round the bundle.

Finally, the cortex of the Psilotaceae shows an absence of large intercellular spaces; in the old stem of *Tmesipteris* it is a dense band of sclerotic tissue, while the layers immediately outside the sheath show that peculiar brown thickening of the walls already described by others [2]. In *Psilotum*, the epidermis, with its thick outer wall, is succeeded by three or four layers of thin-walled chlorophyll-parenchyma, within which is a sclerotic band, and this is again succeeded by a thinner-walled cortex and endodermis.

From these descriptions of the cortex in living Lycopods and Psilotaceae, and in *Lepidostrobus Brownii*, and various stems of *Lepidodendron*, it is clear that these plants show considerable variety of detail in the nature of this tissue; a special point of interest for us is that those different types of structure which obtained in the fossil forms can be matched by similar characters in living forms of close alliance. Thus the curious intertwined filamentous middle cortex of *Lep. fuliginosum* finds its counterpart in the middle cortex of *L. Selago*, though in a less pronounced manner. The case of *Lep. Harcourtii*, where the innermost firm band appears not distinctly differentiated, is matched by *L. nummulariifolium*, or (exclusive of the large air-space round the steles) by *Selaginella Martensii* or *Willdenovii*. But the most interesting for comparison are those with large air-spaces; the case of *Lep. selaginoides*, with the large lacunae and connecting trabeculae in the middle cortex, resembles what is

[1] De Bary, Comp. Anat., p. 430.
[2] Bertrand, loc. cit. p. 482, and Figs. 207–212.

seen on a much smaller scale in *L. inundatum* or in the outer tissue of the cortex in *L. alpinum.* It is interesting to note how tissues further removed from the bundles, or closer to them, may be involved in the trabecular development; thus trabeculae may be independent in the cortex, as in *L. inundatum* (Fig. 14) or *L. alpinum* (Fig. 15), or they may surround the leaf-trace bundle, while the latter is still protected by several layers of connected tissue (Fig. 12), or the cells more directly surrounding the bundle, but constituting an ill-defined sheath, may take part in the trabecular development, as in *Lep. Brownii* (Figs. 5, 6, 7), so that the bundle itself appears suspended in a large cavity. It is a very slight step from such a condition to that so well known around the bundles of the stem of *Selaginella.* Here a definite sheath of cells is involved in the formation of the trabeculae[1], the cells undergoing division after their separation laterally from one another. In *Lep. Brownii* the sheath is a less definite one; the cells appear to undergo a somewhat irregular division before they separate laterally, and the trabecular development is only found around the leaf-trace bundles, not directly around the central stele. There are thus a number of points of difference between what is seen in *Lep. Brownii* and the well-known trabeculae of *Selaginella.* The sequence of examples above quoted nevertheless seems to me to be sufficiently continuous to justify the conclusion, on comparative grounds, that *the trabecular development in Selaginella is a specialized and more definite example of that lacunar development which appears in such various forms and positions in cortical tissues of various other Lycopodinous plants*; in the latter the lacunae are more generally distributed; in *Selaginella* they are localized round the vascular masses; but in all cases they owe their origin to the tissues outside the bundle.

I do not anticipate that the detailed characters of structure of the cortex in these plants can ever be used as arguments of

[1] Treub.; Rech. sur *Selaginella Martensii*, p. 11, Figs. 15, 17.

weight in tracing relationship. The very great variety in detail of the cortex in the living species of *Lycopodium* would suffice to shake confidence in arguments upon such a basis, and to suggest that these details are determined by a relatively direct adaptation. It is, however, worthy of notice how similar the modes of variation have been in the plants of the past to those of their living relatives. The same general type, even of the cortical tissues, runs through all these Lycopodinous forms, though that type is certainly less distinctive than are the leading characters of the vascular tissues. After studying such fluctuating characters as these of the cortex, one returns with renewed confidence to the vascular system. Whatever its differences of detail, the Lycopodinous vascular system is a distinctive one; a comparison of the stele of *Lycopodium Selago*, *Selaginella spinosa*, *Lepidodendron selaginoides*, *Lepidostrobus Brownii*, of *Psilotum triquetrum* and *Tmesipteris truncata*, shows a unity of plan which cannot be missed; occurring, as this does, among plants which have always been classed together, the unity of general vascular plan must be accepted as strengthening their relationship. Perhaps the most interesting case of all is the similarity between *Lepidostrobus Brownii* and the Psilotaceae, depending upon the points already noted above. It is to be remarked that while Brown's cone compares with *Psilotum* as regards its endodermis, it approaches *Tmesipteris* in the soft central parenchyma; again, the similarity between the axis of Brown's cone and that of *Selaginella spinosa* is interesting, though not so close in detail as the above; for, in addition to the details of the vascular tissue, there is similarity in the existence of the trabeculae, though not in their distribution. Again, many of the *Lepidostrobi* are proved to be, like *Selaginella*, heterosporous, though this proof is not present for Brown's cone, which only bears microsporangia; it is, however, an obviously incomplete cone, so that the absence of megaspores does not prove it homosporous. Lastly, like *Selaginella*, certain *Lepidodendra* have been shown to possess a ligule; I have not, it is true, been able to see any ligule in

the sections of Brown's cone, but at least it is present in closely allied plants. All these considerations serve to draw the Lycopodinous plants of the present and the past more closely together as a natural family, while it is interesting to note that the lines of similarity as regards structure, form, heterospory and details of sporangia, do not focus themselves specially between any two genera, but are such as to suggest complex cross-relationship between the several representatives of this very natural series.

When we pass from the vascular tissues to the study of the sporangia of these several forms, differences are to be noted. I have shown elsewhere [1] that there is considerable variety in form and complexity of the sporangium within the genus *Lycopodium*; but, while more or less extended and curved in a plane tangential to the axis, the sporangium does not in any case extend far along the leaf in a radial direction from the axis. The sporangium of *Lepidodendron*, however, differs in not being strongly curved in the tangential plane, while it is extended largely in a radial direction; trabeculae of sterile tissue have been found rising from the base of the sporangial cavity and extending far up into the sporogenous mass. There can be no doubt of the homology of the sporangium of *Lepidodendron* with that of *Lycopodium*. But when the sporangium of *Lepidostrobus Brownii* is compared with that of *Tmesipteris* or *Psilotum* a question of the homology may arise. On grounds of internal development of the synangia in *Psilotum* and *Tmesipteris*, which are stated elsewhere [2], I was led to agree with the conclusion of Graf Solms from external observation of development in *Psilotum*, viz. that the whole sporangiophore is of foliar nature, and that the synangium is a growth from its upper surface. There is also a general similarity in form between the synangium of *Tmesipteris* and the sporangium of *Lepidostrobus*. Thus the *whole synangium* of *Tmesipteris* would correspond in position and form to the sporangium of *Lepidostrobus*, that is, a body with two or three

[1] Proc. Roy. Soc., no. 304, vol. 50, p. 267, &c., 1892.
[2] Proc. Roy. Soc., no. 321, vol. 53, p. 19, 1893.

loculi would correspond to a unilocular one. I therefore put forward the hypothesis that such a sporangium as that of *Tmesipteris* might be derived by partitioning from a sporangium such as that of *Lepidostrobus*, this hypothesis being in accordance with the observed details of development. Now the observations detailed above show that there is very close similarity between the vascular tissues of *Lepidostrobus* and those of the Psilotaceae; and this evidence, coming as it does from a somewhat different quarter, appears to me to give material support to the hypothesis. On anatomical grounds the affinity of the Psilotaceae is clearly Lycopodinous; all other Lycopods show uniformity in the general plan of the strobilus. These considerations should weigh with morphologists in favour of the theory that the synangium of the Psilotaceae is homologous with the sporangium of other Lycopods, that the sporangiophore is a branched leaf homologous with the simple sporophyll of *Lycopodium* or *Lepidostrobus*, and that the single fruiting axis of *Tmesipteris* or *Psilotum* is comparable to a lax Lycopodinous strobilus.

EXPLANATION OF FIGURES IN PLATES XVI AND XVII.

Illustrating Professor Bower's paper on *Lepidostrobus Brownii.*

Fig. 1. Transverse section of the axis of Brown's cone. ×4.

Fig. 2. The same in median longitudinal section. ×4.

Fig. 3, *A*. Part of a transverse section, showing (*p*) the central parenchymatous pith; *xy*, the wood; *c*, the innermost band of cortex; *sh*, the endodermis (?). ×300.

Fig. 3, *B*. Part of transverse section of the central stele of *Psilotum*. ×150.

Fig. 4, *A*. A leaf-trace bundle of *Lepidostrobus Brownii*, embedded in the dense innermost band of the cortex (*c*). The cells marked × have divided parallel to the surface of the bundle, and elongated slightly. Compare Figs. 5, 6. The arrow in this and other figures points towards the centre of the axis. ×300.

Fig. 4, *B*. Section of another leaf-trace bundle to show how variable the details of structure may be from that shown in Fig. 4 (*a*). ×300.

Fig. 4, *C*. Transverse section of a leaf-trace of *Lycopodium Phlegmaria* for comparison with those of *Lepidostrobus Brownii*. ×300.

Fig. 5. Cells × adjoining a bundle (in *L. Brownii*) which is approaching the periphery of the innermost band of cortex: they have divided, and have elongated so as to form intercellular spaces (*o*). ×300.

Fig. 6. Ditto: the intercellular spaces (*o*) are larger, and cells more elongated, so as to form trabeculae. ×200.

Fig. 7. Transverse section of a leaf-trace bundle with more fully-formed trabeculae traversing the large space which almost surrounds the bundle. The position of this bundle was in the outer dense cortex. ×300.

Fig. 8, *A*. A single bundle isolated in the large cavity of the middle cortex, but showing on its margin points (×) where the trabeculae have been broken away. Elongated trabeculae (*tr*) project towards it from the dense peripheral band of the cortex. ×300.

Fig. 8, *B*. Another similar bundle. ×300.

Fig. 9. Median longitudinal section of such a bundle, showing (×) points where trabeculae have broken away. ×300.

Fig. 10. Radial section, including three vascular bundles, together with the tissue surrounding them, and the trabeculae, many of which have broken away, from near the apex of the cone.

Fig. 11. A few trabeculae, more or less broken, from a longitudinal section drawn on a larger scale. ×150.

Fig. 12. Transverse section of a leaf-trace bundle of *Lepidodendron selaginoides* (slide No. 38) in the middle cortex, surrounded by lacunae traversed by irregular trabeculae. ×150.

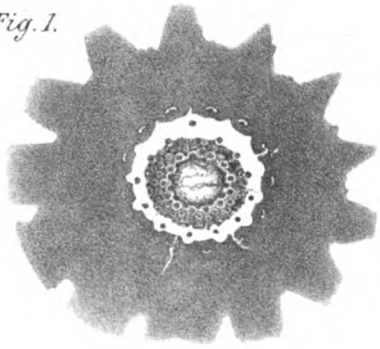
Fig. 13. Part of middle cortex of *Lepidodendron fuliginosum* (slide No. 12), showing irregular intertwined filaments. ×70.

Fig. 14. Small portion of the middle cortex of *Lycopodium inundatum*, showing trabeculae. ×150.

Fig. 15. Cortex of *Lycopodium alpinum*. ×70.

Fig. 16. Small part of the periphery of the stele of *Lycopodium dichotomum*, showing how at certain points, though not continuously, the endodermis (*e*) may be represented by a single series of cells.

Fig. 1.



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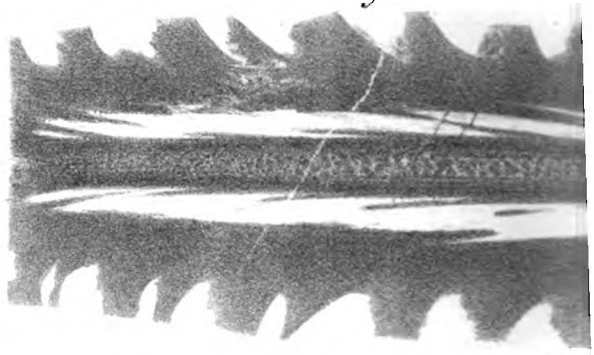


Fig. 3.A

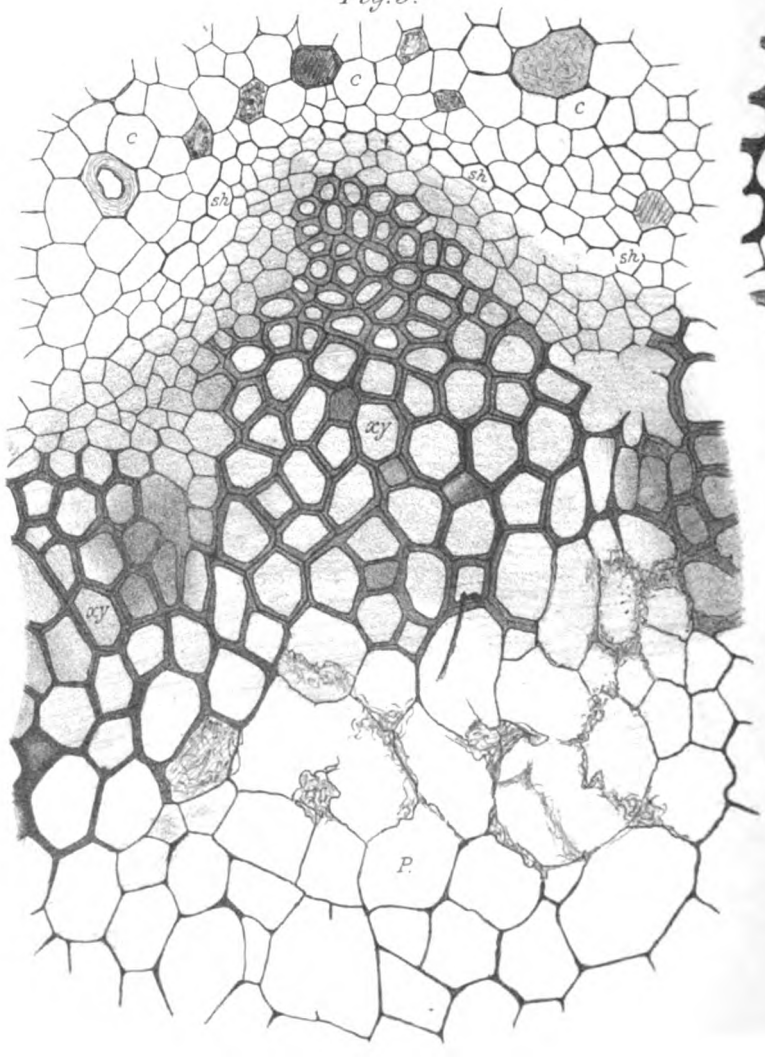


Fig. 4.B

BOWER. — LEPIDOSTROBUS.

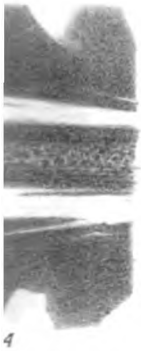
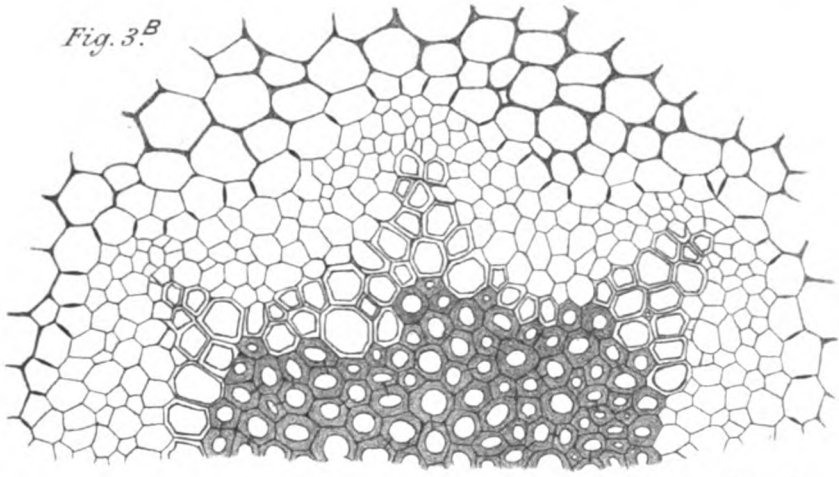


Fig. 3.[B]



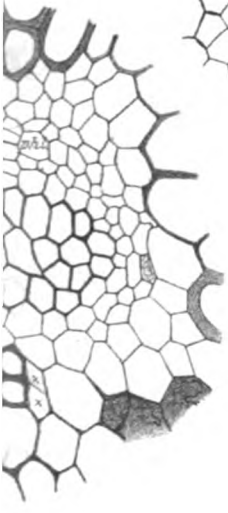
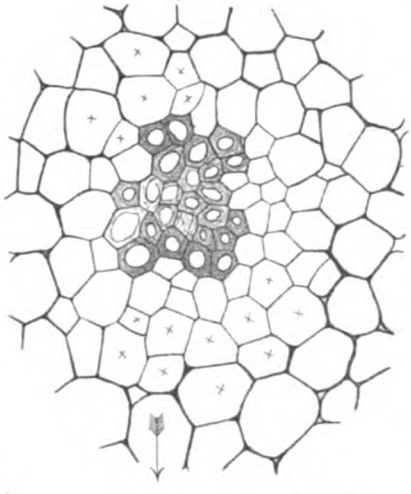


Fig. 4.[C]



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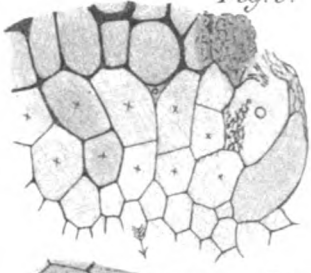

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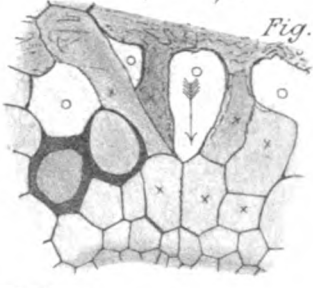

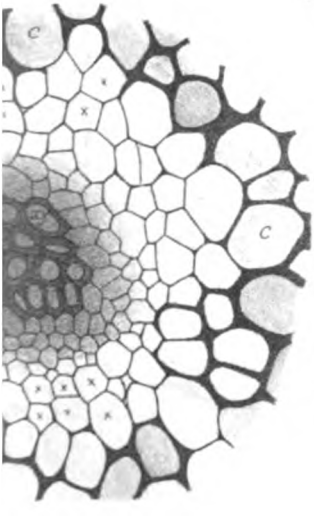

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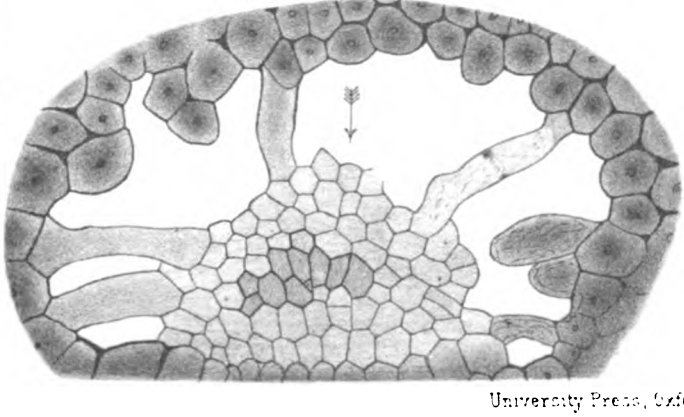

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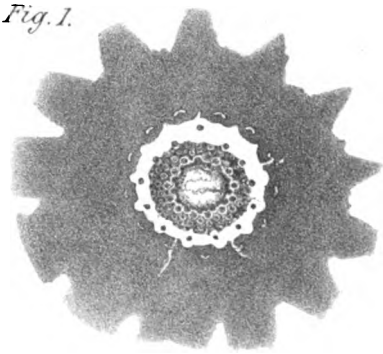

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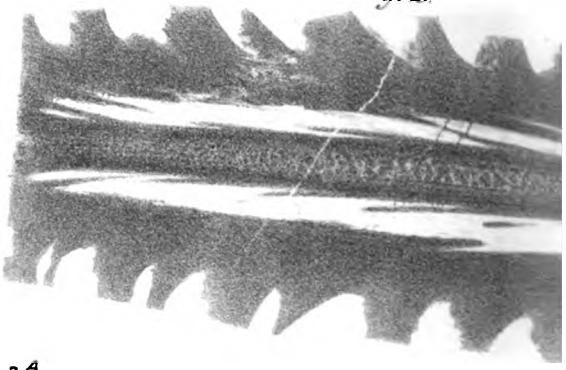

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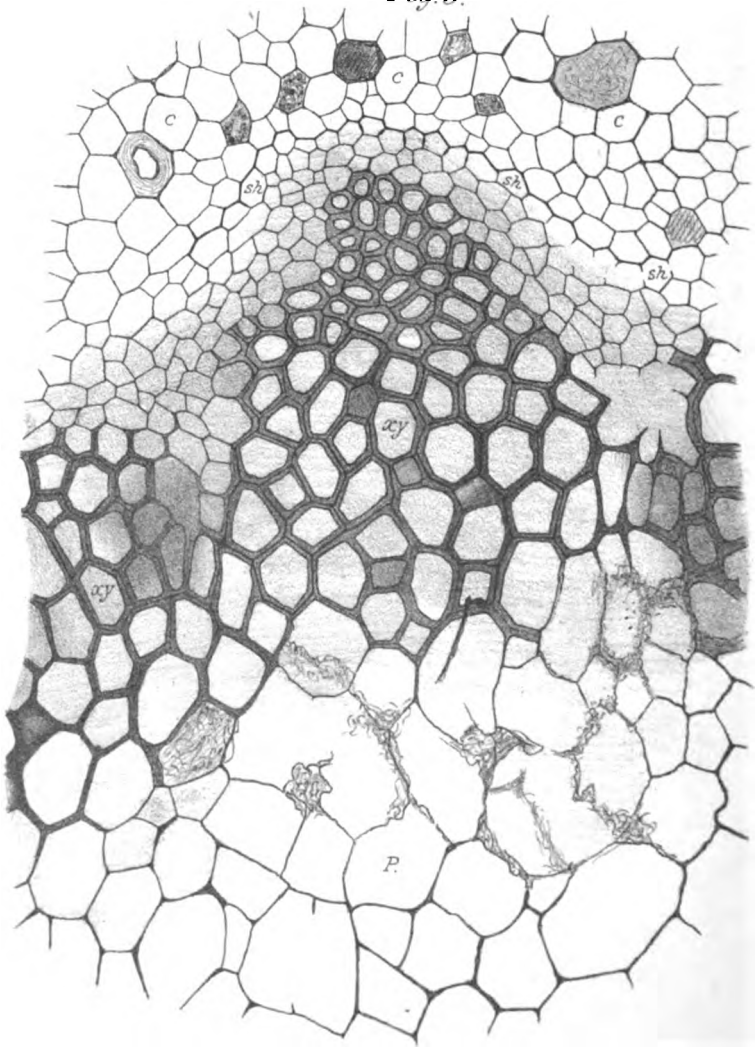

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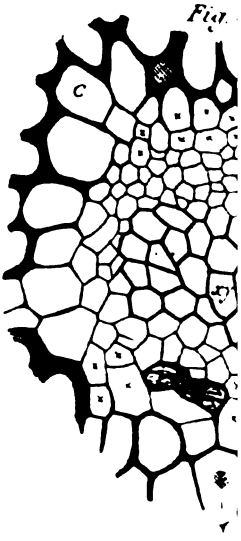

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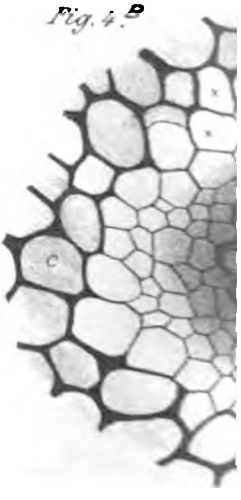
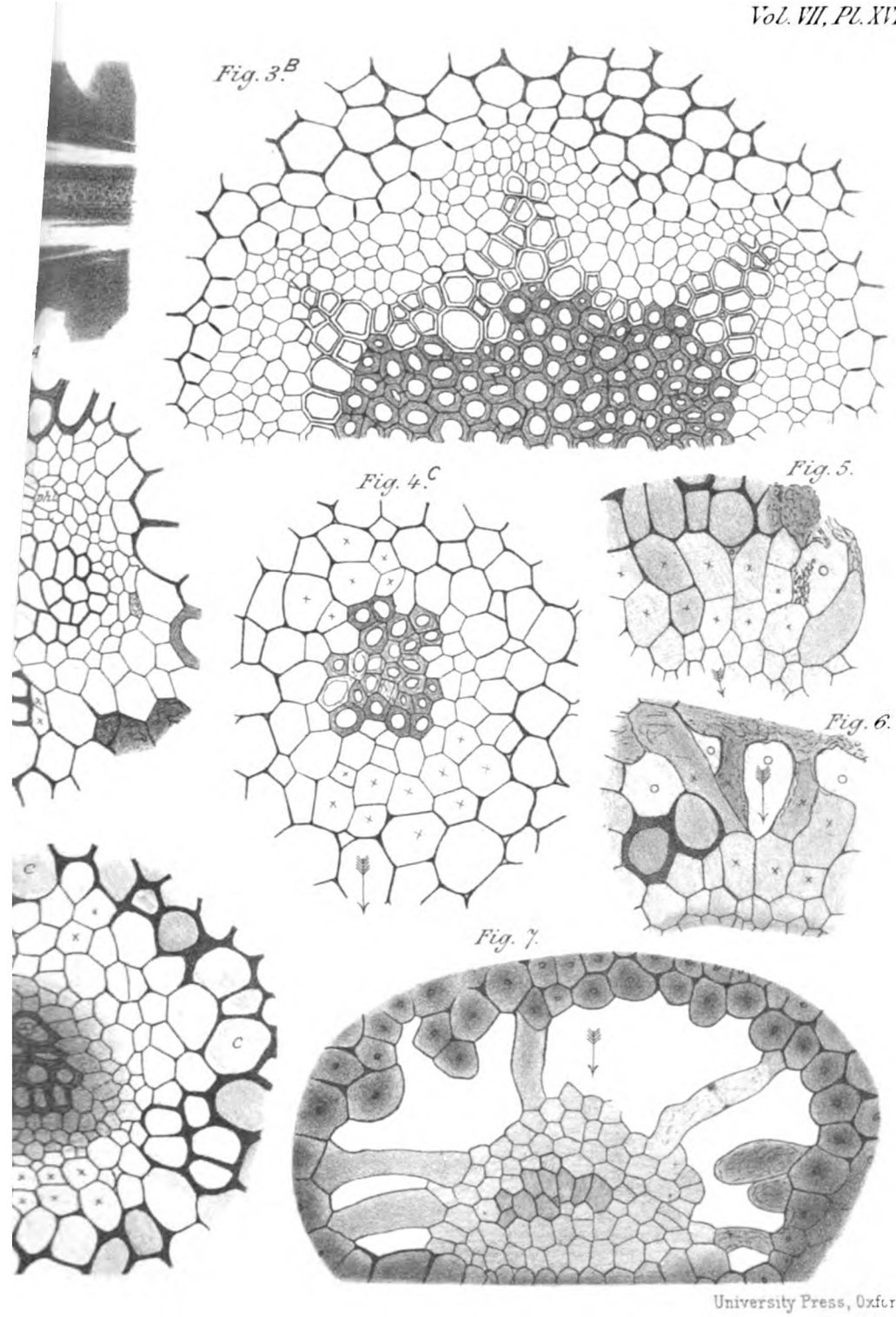


BOWER. — LEPIDOSTROBUS.



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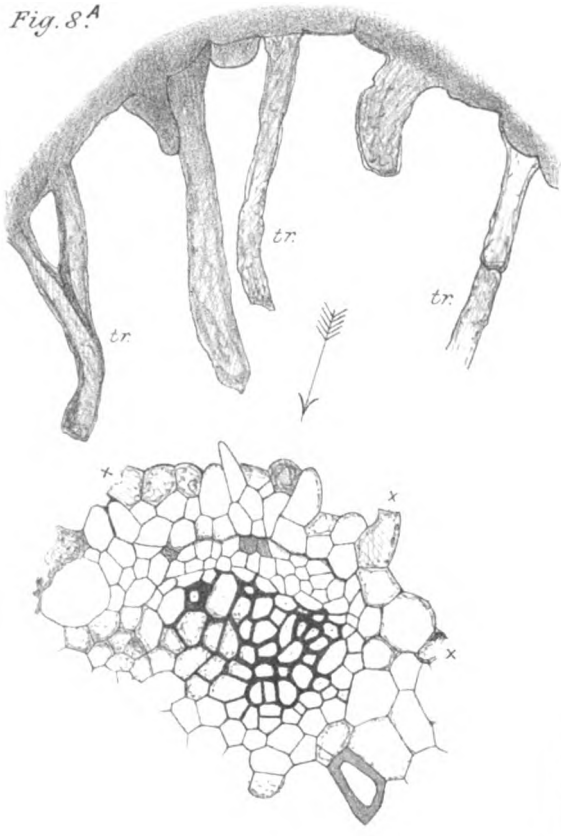

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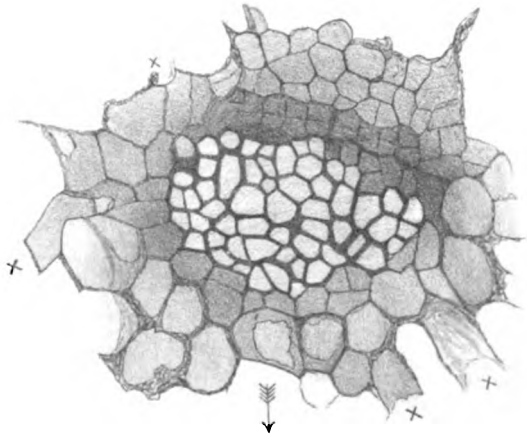

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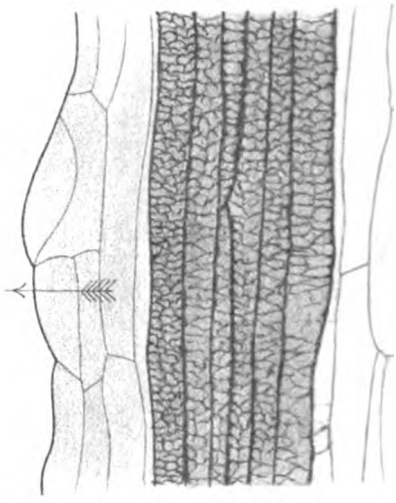

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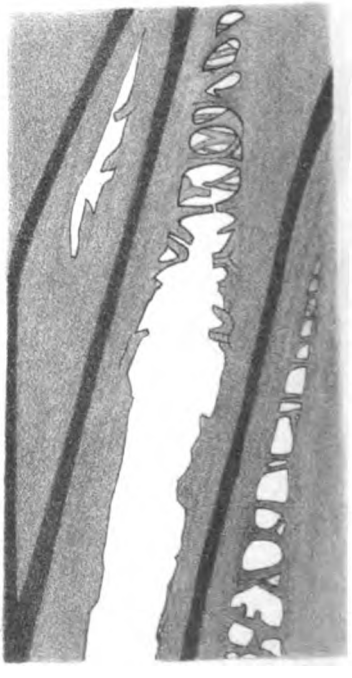

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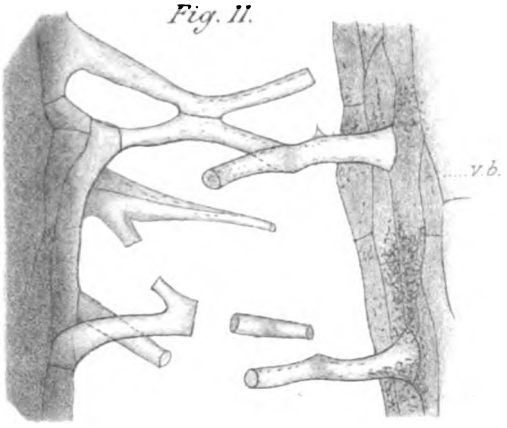

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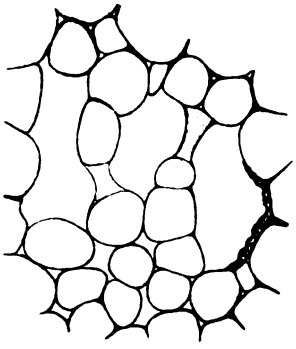

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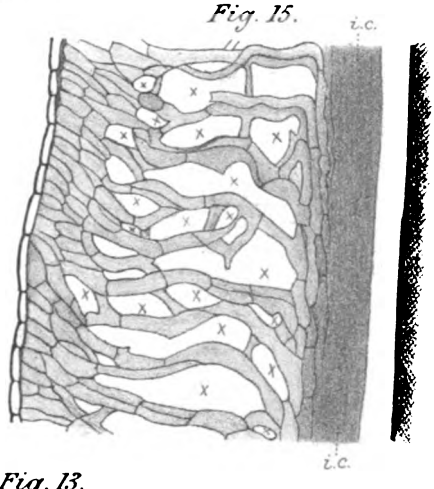

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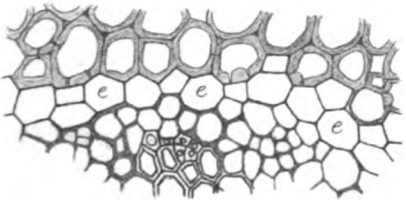

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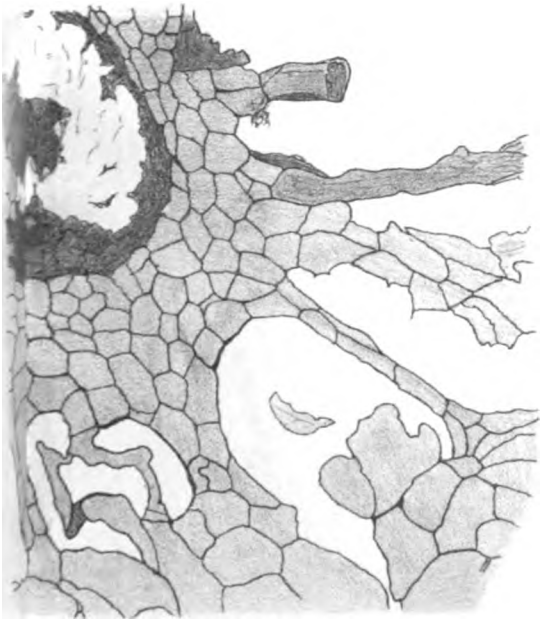

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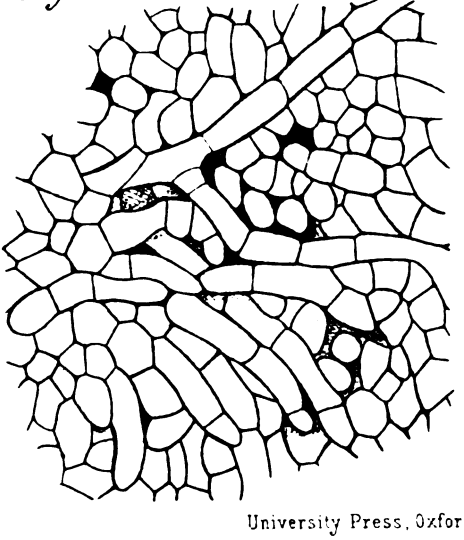

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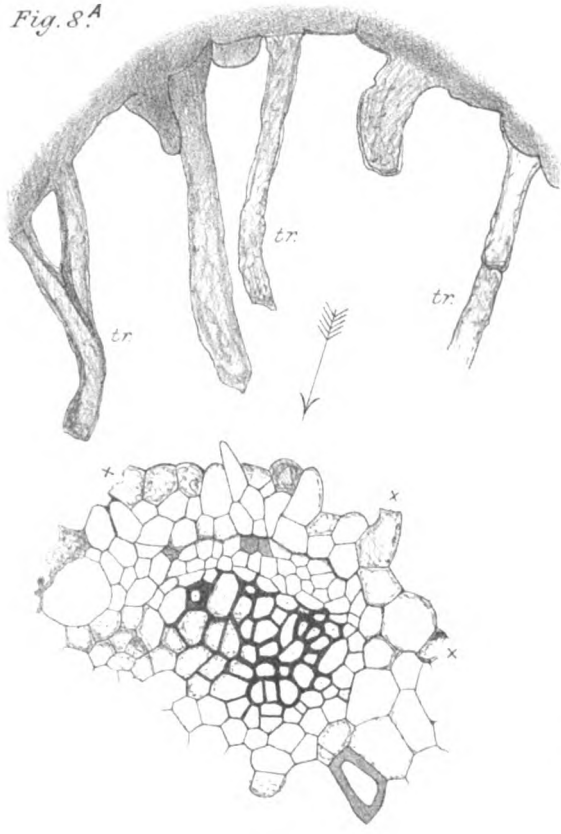

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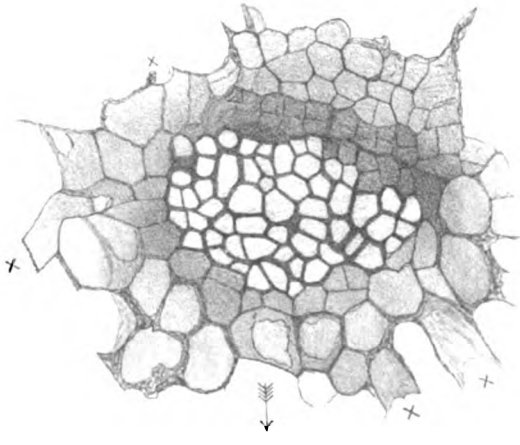

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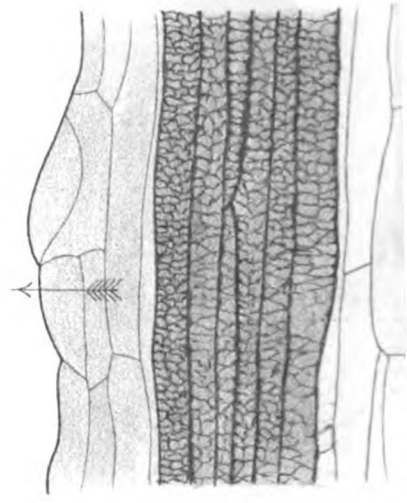

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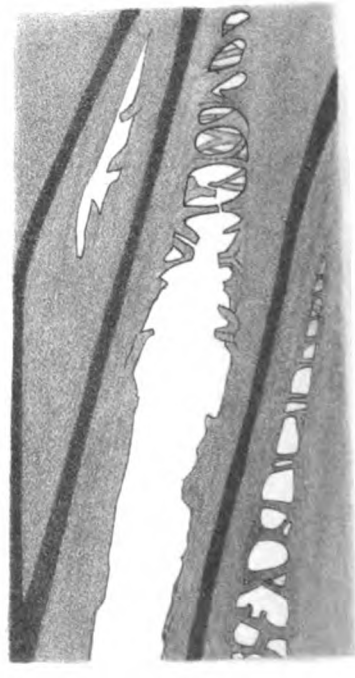

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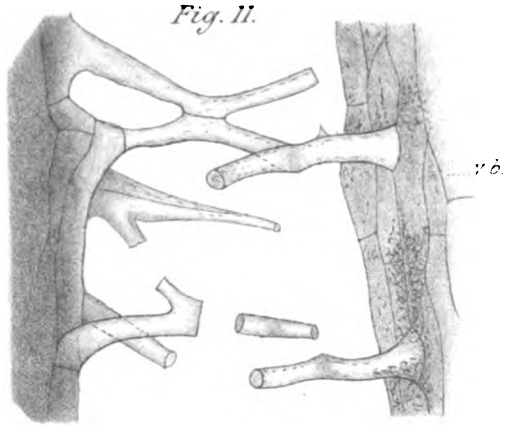

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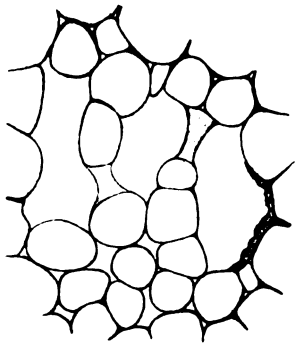

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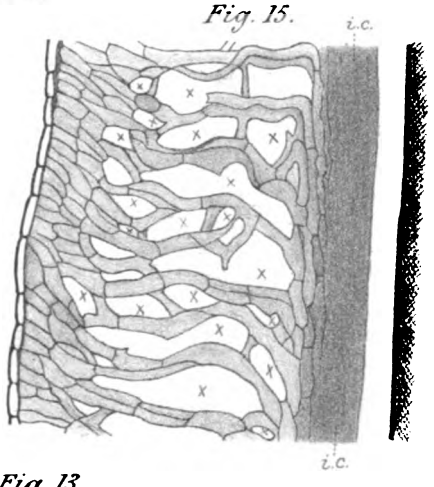

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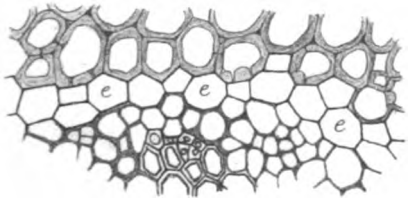

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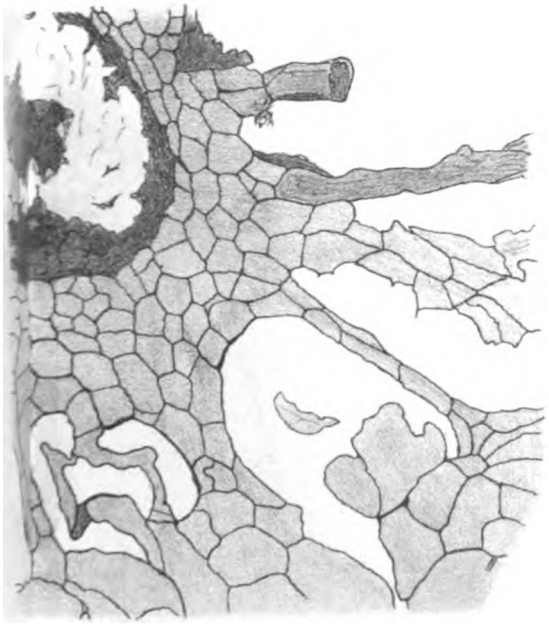

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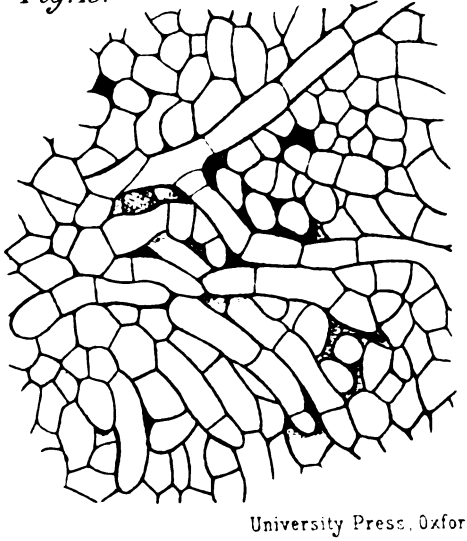

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On the Siliceous Deposit in the Cortex of Certain Species of Selaginella, Spr.

BY

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With Plate XVIII.

THE existence of a mineral incrustation on the cortical wall of the lacuna of *Selaginella Martensii*, Spr. is well known to botanists; but, so far as I am aware, no account has been published of the exact nature, distribution and mode of origin of this mineralization, nor has its presence in any other species of the genus been noted. In discussing the structure of the stele in *S. Martensii*, Strasburger[1] casually mentions the presence of the deposit, but gives no details.

Whilst engaged last winter on a research into the comparative anatomy of the genus *Selaginella* in the Botanical Laboratory of the University of Strassburg, Graf zu Solms suggested to me that it might be worth while to examine the deposit in some detail, and to determine its exact chemical character, distribution, and mode of origin in the species, and also to investigate whether similar deposits were to be met with in other species of the genus. I have examined in all fifty-two species of *Selaginella*, for material of which I am indebted to

[1] Ueber d. Bau. u. d. Verricht. d. Leitungsbahnen in d. Pflanzen: 'Hingegen sind an letzteren oft unregelmässige, spröde, farblose Belege zu sehen, die Kieselsäure zu sein scheinen.'

Graf zu Solms; to the Director of the Royal Gardens, Kew; and to the Curator of the Botanic Gardens, Glasnevin, Dublin. Of these species I found sixteen to contain a siliceous deposit, viz.: *S. Martensii*, Spr. (both the type species and the varieties *flexuosa*, *compacta*, *stolonifera* and *variegata*); *S. grandis*, Moore; *S. Griffithii*, Spr.; *S. inaequalifolia*, Spr.; *S. Lobbii*, Moore; *S. haematodes*, Spr.; *S. suberosa*, Spr.; *S. atroviridis*, Spr.; *S. erythropus*, Spr.; *S. bakeriana*, Bail.; *S. stenophylla*, A. Br.; *S. involvens*, Spr.; *S. gracilis*, Moore; *S. flabellata*, Spr.; *S. caulescens*, Spr. var. *amoena*, and *S. emiliana*.

In all these the deposit presents the same essential characters; but there are individual differences, more especially in the distribution and amount of mineralization. I purpose selecting for detailed description and analysis *S. Martensii*, Spr., var. *compacta*, A. Br. (a form kindly named for me by Professor Dr. Kuhn), and giving a brief account of the deposit as seen in the other species.

S. Martensii, Spr., var. **compacta**, A. Br.

Without going into the minute histology of the stem, which will be treated of fully in a future paper, it will be sufficient to say that the single stele is suspended in a well-developed lacuna by trabeculae stretching from the innermost layer of the cortex to the pericycle. The external surface of the pericycle is covered by a cuticle. The trabeculae are of two kinds, simple and compound. The simple type consists of usually one, at most three cells; the compound type, which is by far the more numerous, of an attaching cell on the one side to the pericycle—endodermal cell—on the other to the cortex, and between these a cluster of thin-walled swollen cells, containing protoplasm, chlorophyll and starch. In the fully-developed stem these swollen cells completely fill the lacuna, and give it the appearance of a layer of loosely-arranged parenchyma, with numerous intercellular spaces. The cortical wall of the lacuna is covered by a plentiful deposit of mineral matter, laid down apparently in quite irregular colourless plates. If a portion of a stout stem be longitudinally sectionized so as

to expose the inner surface of the cortex, and if the section be cleared, one gets an appearance similar to that represented at Fig. 1, Pl. XVIII. The vertical walls of the innermost cortical cells are observed more or less clearly through thin glassy plates of quite irregular size and shape. They have occasionally smooth, but more commonly very ragged edges, from which shorter or longer cracks extend into the body of the plate. Frequently a large plate becomes cracked into several pieces, the cracks widening into narrow channels. The edges of these channels are as a rule sharply defined, but occasionally at their inner extremities they become very faintly marked, as though the plates at that point were of extreme thinness. This feature is represented in the central plate of Fig. 1. Here and there areas occur which are quite uncovered by the deposit, but it is possible that in these situations the plates have been displaced in the preparation of the section. In a longitudinal section of the stem, prepared to show the edge of the plates, one finds that the above interpretation of the surface-view is the correct one, for the plates are seen to be of irregular thickness, in some places being as much as three times the thickness of the cell-wall they cover, in other places lessening in thickness very considerably and finally ending in an exceedingly delicate film, only distinguishable by its difference in refractive index from the cell-wall below. Fig. 2 shows a portion of such a section. Through gaps left in the deposit the trabecular cells arise from the cortex; the base of one of these is shown in the figure.

In addition to the deposit to be found on the outer lacunar wall, there occurs what seems to be a similar mineralization both in the walls of the innermost cortical cells and on the swollen cells of the compound trabeculae. If a transverse section be prepared cleared and mounted in balsam, the deposit may be quite easily made out in both situations. In Fig. 6 one of the compound trabeculae of *S. Martensii*, var. *stolonifera*, is represented. (The lower end of the figure is that next to the cortex, the upper is that next to the pericycle.) The mineral deposit in such a trabecula occurs in

the small intercellular spaces between the constituent cells of the median cluster. It fills up these spaces completely and spreads as a very delicate film over the walls which are adjacent to each other. I can find no evidence of mineralization on the outer sides of these cells, facing the lacuna proper. Moreover, if the trabeculae be destroyed by concentrated sulphuric acid, the mineralization remains, and has the form of a number of concave shells or hemispheres united by their bases. Sometimes the basal cell next the cortex has a mineral deposit running up its external wall for a short distance, more commonly the deposit ceases abruptly at the base. I have never found any deposit on the pericycle nor on the cuticularized trabecular cells arising from it. Further, the cortical cells have a mineral deposit in their walls continuous with the surface layer in the lacuna. The small intercellular spaces between the cells of the innermost cortex are filled with the same deposit. As a rule, in var. *compacta*, only the three cell-layers next the lacuna are so mineralized; the cell-walls of the outer cortex contain no mineralization (Fig. 5).

As one would expect, the younger branches have considerably less development of the mineral, although it can be traced right up the stem almost to the merismatic region, being coincident in its appearance with the lacunar space. Fig. 3 shows a surface-view of the cortex lining the lacuna of a young branch about a quarter of an inch from its apex. Here it will be seen that the plates appear to arise at first close to or immediately over the vertical walls of the cortical cells. Two types of plates may be distinguished at this stage: (1) those which arise immediately over the vertical walls and spread out equally to either side, and (2) those which arise to one side of the vertical wall and develop over the cell-surface on that side only. The former are generally oblong and rather narrow, thickest just over the vertical wall and thinning off to either side. In sectional outline they appear as extremely obtuse-angled triangles. The second type of plate is thick at one (the outer) side, and thins off towards the centre of the cell. These plates have the appearance of razor-blades.

Cracks very soon appear on the thickened edge, passing inwards towards the centre of the adjoining cell, often bifurcating or branching irregularly as they go. The thin edge very often, even in the young condition, extends quite across the cell. If a development of the second type of plate occurs on the same cell from both or all sides, the deposits may become continuous in the centre of the cell area. The very youngest condition of the deposit I have been able to detect give the appearance of exceedingly delicate rods lying just over the vertical cell-walls. I have never seen the plates begin from the centre of the cell-area.

An examination of many sections taken from different parts of the stem leads one to the conclusion that the cracks are secondary in origin, and that the differential growth of the cortex obliterates in time the evidence of the mode of origin of the plates. It will be seen that in Fig. 1 the plates bear no relation whatever to the vertical cell-walls, whilst in Fig. 3 the relationship is obvious. A comparison of the size of the superficial cortical cells renders this still more apparent.

If a suitable section be boiled in concentrated sulphuric acid the organic matter is destroyed, but the plates remain quite uninjured. Figs. 4 and 7 show several forms of plates—Fig. 4 from a young stem, Fig. 7 from a mature stem. The trabeculae must of necessity be formed before the commencement of the deposition. The deposit, indeed, completely surrounds the base of the trabeculae, but does not, at least as a general rule, run up them. Fig. 7 represents a large plate from a mature stem, showing two apertures through which trabeculae passed in the fresh condition.

With regard to the chemical nature of the deposit there cannot be a difference of opinion, although it is not quite so easy to feel certain as to its mode of origin and deposition. The plates are undestroyed by concentrated hydrochloric, nitric, or sulphuric acids, hot or cold. They are unaffected by heating to redness. They cannot be stained, and they remain after the sections have been treated with cupric ammonium hydrate. All these negative reactions point to the

mineral being what it has always been stated to be, viz. silica.

The amount of silica present in measured lengths of the stem was next determined. Portions of stem were dried for twenty-four hours over strong sulphuric acid. The dried material was then fused over a blowpipe and the weight of ash determined. It was found to amount to 9 per cent. of the dry weight.

The ash was then fused over the blowpipe with excess of sodium carbonate. The fusion was boiled with water, and after the addition of a little hydrochloric acid the whole mass was evaporated just to dryness on a water bath. Hydrochloric acid was again added, and the mixture taken again just to dryness on the water bath.

Water and a few drops of hydrochloric acid were then added, the whole slightly warmed and allowed to stand for an hour. The separated SiO_2 was filtered off and weighed. It was found to amount to 30 per cent. of the ash.

To the hot filtrate, ammonium chloride, ammonium hydrate and ammonium oxalate were added. After twenty-four hours the precipitate was filtered off, and, to ensure separation of the calcium and magnesium, the precipitate was redissolved in hydrochloric acid preparatory to a second precipitation with ammonium oxalate. A small quantity of SiO_2 (3 per cent. of the ash) remained here undissolved. The calcium was reprecipitated by ammonium hydrate and ammonium oxalate and filtered off after twenty-four hours. The calcium oxalate was then transformed into calcium oxide by ignition and weighed as such. It was found to amount to 18 per cent. of the ash.

The filtrate was then treated with ammonium hydrate and ammonium phosphate and allowed to stand for forty-eight hours. The magnesium was thus precipitated as $Mg\,NH_4\,PO_4$. The precipitate was filtered off and the magnesia determined from the weight of $Mg_2\,P_2\,O_7$, obtained by heating. It was found that the magnesia amounted to 18·7 per cent. of the ash.

The other ash constituents were not determined. It may be concluded from this analysis that SiO_2 is taken up by

the plant as a soluble silicate of magnesia or of lime, or possibly as a double silicate of these bases. (I am greatly indebted to my colleague Dr. T. L. Bailey for his aid in this part of my work.)

S. Martensii, var. **flexuosa.**

In this variety the deposit on the lacunar wall is very well marked and the plates are of considerable thickness. The superficial cortical cell-layer alone, however, is well mineralized, although here and there the mineral may be detected in the minute intercellular spaces between the second and third layer of cortical cells. On the other hand, the trabeculae are plentifully supplied with $Si O_2$, the plates lying loosely round the basal cells next the cortex, and the clustered cells of the compound trabeculae have a large amount of $Si O_2$ in their intercellular spaces.

S. Martensii, var. **variegata.**

In this form the trabeculae are not so much silicified. The plates on the lacunar walls are more uniform and thinner, but the mineral can be traced into the cortex to the third, or even, in some places, the fourth cell-layer.

S. Martensii, var. **stolonifera.**

This variety resembles in all respects, so far as regards the siliceous deposit, var. *flexuosa.*

S. grandis, Moore.

I am not aware of any published account of the occurrence of $Si O_2$ in any species of *Selaginella* save *S. Martensii.* In examining the fine collection in cultivation at the Royal Gardens, Kew, I found that a deposit of the same mineral occurred in the cortex of *S. grandis*, as well as in the other species named above. I propose to give a brief account of the essential features shown by the deposit in that form.

In the first place the deposit is relatively much greater in amount than in *S. Martensii* or any of its varieties.

The plates are thicker and more regular in form, but still have the same characters so far as regards their mode of

origin, viz. the first appearance of the deposit is over the vertical walls of the cells of the innermost layer of the cortex. The knife-blade form of plate is by far the most frequent, though occasionally the double-wedge form is to be met with.

In older stems the deposit is on the whole extremely regular, and consists of ragged, much-elongated bands, which anastomose frequently, leaving gaps between for the exit of trabeculae. Fig. 8 shows this feature in the siliceous lining in the main stem after treatment with concentrated sulphuric acid.

The full-grown plant has a well-marked primary axis, quite unbranched for a considerable distance, finally deliquescing in a flabellate manner into a number of secondary axes. At the point of origin of a branch the mineralization is much more irregular. Fig. 9 shows a portion of the deposit in such a situation after treatment with sulphuric acid. The silica runs up the trabecular cells and is deposited also between the cells of the compound trabeculae.

The cells of the inner cortex are long and sclerotized, have narrow lumina and run longitudinally. The cortical cells of the trabeculae also run in a creeping manner along the cortical wall before crossing the lacuna. In section the silica is found to penetrate between these cells, so that the trabecular cell as it leaves the cortex is encased by the mineral. Fig. 10 shows a section of the cortex next to the lacuna, where it will be seen that the silica is in places pierced by apertures through which run the creeping cortical cells. Silica may also be distinguished in the minute intercellular spaces between the cortical cells.

S. Griffithii, Spr.

The siliceous deposit in this species has similar characters to that in *S. grandis*, although it is much smaller in amount. The plates in the adult stem are not so regular, but the cortical trabecular cells are, at their origin from the cortex, encased in SiO_2.

S. inaequalifolia, Spr.

In young stems the deposit is small in amount and can scarcely be said to do more than form a thin lining to the cortical wall of the lacuna, though here and there it penetrates between the cells of the innermost layer of the cortex. In old stems, however, the appearance presented by the deposit is very similar to that seen in *S. grandis*. As this species is very commonly used for laboratory study, perhaps I may add that the deposit is very clearly seen in sections mounted in balsam. The figure from Sachs' Text-book, quoted in De Bary's Comparative Anatomy of Phanerogams and Ferns, and indeed in most other text-books treating of the genus, represents no such deposit. As a matter of fact the figure is more fatally erroneous in other respects—for instance, three steles are represented, each with two protoxylem-masses, one at either margin; whilst in reality there are twelve to fifteen protoxylems (in a full-grown stem), each stele having at least four. The two lateral steles are also occasionally double, two within one lacuna. I need not, however, discuss the anatomy further in the present paper.

S. Lobbii, Moore.

In the large suberect stems of this species a small amount of $Si\,O_2$ may be distinguished lying on and round the innermost cortical cells. The quantity is very small compared to that in some of the other species described. There are no special features of the deposit in this form that require description.

S. haematodes, Spr.

The thick-walled sclerotic cells lining the cortical wall of the lacuna have a small incrustation of $Si\,O_2$, which penetrates between the cells, but is not traceable beyond the third layer. There is no deposit on the swollen parenchyma, which partially fills the lacuna.

S. suberosa, Spr.

The deposit in this species is also small in amount and

generally consists of a simple coating of thin irregular plates over the parenchyma forming the cortical wall of the lacuna.

S. atroviridis, Spr.

The features of the deposit in this species are precisely similar to those of *S. Martensii*, var. *compacta*.

S. erythropus, Spr.; **S. bakeriana**, Bailey; and **S. stenophylla**, A. Br.

A thin deposit occurs in these species on the innermost cell-layer of the cortex, penetrating between its constituent cells.

S. involvens, Spr.

The cortex of this species shows considerable variation from the type condition. The epidermis encloses several layers of sclerotized elongated cells with minute intercellular spaces, showing a gradual transition to the loosely arranged inner-layer and finally to what corresponds to the trabecular tissue. In fact, the entire conjunctive tissue outside the pericycle and endodermis cannot be differentiated into layers such as one sees in other forms. The intercellular spaces are numerous and large and either filled or lined by a large amount of siliceous deposit. The silica is most abundant in the middle layers, but can be traced almost to the epidermal layer outwards, and to the endoderm inwards. In the latter case the deposit begins in the angles formed by the juxtaposition of the cylindrical cells, of which the cortex is uniformly composed.

S. gracilis, Moore.

The deposit in this species resembles in all respects that in *S. Lobbii*.

S. flabellata, Spr.

The silica is confined to the minute intercellular spaces between the two innermost layers of the cortex and to the depressions between the free cells of the superficial layer facing the lacuna.

S. caulescens, Spr. var. **amoena**, and **S. emiliana.**

The very faintest trace of silica is to be found in the minute intercellular spaces between and on the innermost cortical cells. I could detect none, however, in *S. caulescens* itself.

Before any definite conclusions can be arrived at as to the part played by the siliceous deposit in the economy of the species that possess it, it will be necessary to cultivate specimens in an artificial soil from which silicates have been carefully excluded. In the absence of any experimental data on this aspect of the question, I can only at present suggest that the SiO_2 is an excreted product, and that calcium and magnesium are absorbed, at least in great part, in the form of soluble silicates, the silica being eliminated in the insoluble form. What the agent in the decomposition is would also form an interesting question. I have endeavoured to artificially produce decomposition of the silicate by hermetically sealing branches of *S. Martensii* in a glass tube filled with water saturated with carbon dioxide. After a week's exposure, however, I could detect no trace of silica in any situation save where it is to be found in the fresh condition.

EXPLANATION OF FIGURES IN PLATE XVIII.

Illustrating Mr. Harvey Gibson's paper on Siliceous Deposit in *Selaginella.*

All the figures are drawn under a magnification of 350.

Figs. 1–5. *S. Martensii*, Spr. var. *compacta.*

Fig. 1. Surface view of the siliceous plates from the cortical wall of the lacuna of a fully-developed stem.

Fig. 2. Siliceous plates on the cortical wall of the lacuna in section.

Fig. 3. Young stages in the development of the plates, taken from the stem about a quarter of an inch from the meristem region.

Fig. 4. Isolated plates from the apex of a young stem after treatment with concentrated sulphuric acid.

Fig. 5. Transverse section though the cortex after clearing in eau de Javelle and mounting in balsam. (The dark shading indicates the siliceous deposit.)

Fig. 6. Trabecula of *S. Martensii*, var. *stolonifera*, showing distribution of silica between the median cells of the trabecula.

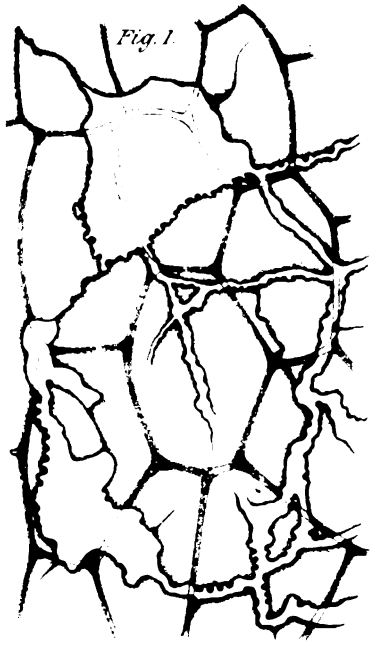
Fig. 7. Isolated siliceous plate from a mature stem of *S. Martensii*, var. *flexuosa*, showing two apertures for the exit of the basal cells of trabeculae.

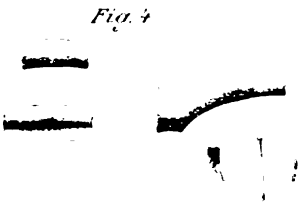
Figs. 8–10. *S. grandis*, Moore.

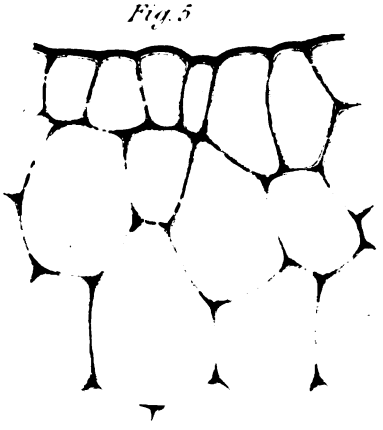
Fig. 8. Siliceous plates from the cortical wall of the lacuna of the mature stem at the level of an internode.

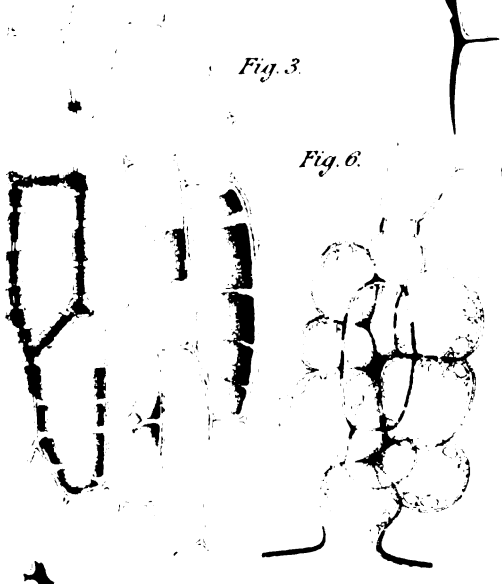
Fig. 9. Siliceous plates from the cortical wall of the lacuna at the origin of a branch.

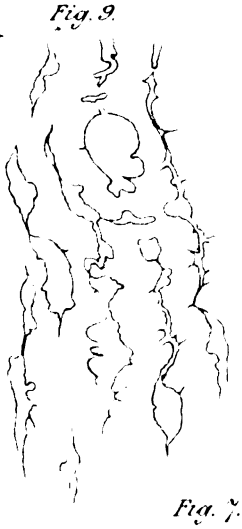
Fig. 10. Transverse section of the inner cortex of the mature stem. The dark shading indicates the siliceous deposit.

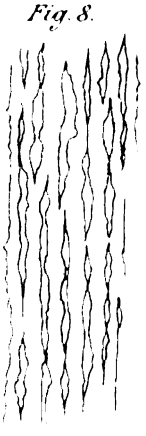












Harvey Gibson del. University Press, Oxford

HARVEY GIBSON.— SILICEOUS DEPOSIT IN SELAGINELLA.

A Criticism, and a Reply to Criticisms.

BY

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IN a recent number of the Annals of Botany[1] Professor Goebel has given an epitome[2] of his interesting observations on the sexual generation of *Buxbaumia*, recognizing in it the simplest known type of a moss, and pointing out that it 'very nearly comes up to the hypothetical ideal of the simplest primitive moss' which he had suggested elsewhere[3]. I do not wish to contest the conclusion thus worded. Certain of the points brought forward should, however, suggest caution before accepting the above quotation as more than a plain statement of fact. When Professor Goebel proceeds (p. 357) to conclude 'that *Buxbaumia* is an ancient type of moss which still retains a number of primitive characters,' he enters ground which is more open for debate: it will be necessary, before accepting this conclusion, to decide whether the characters upon which it is based are really relatively primitive or the result of reduction.

There seems good reason to think that reduction has had its influence upon *Buxbaumia*: Professor Goebel himself draws attention to the absence of chlorophyll from the solitary pro-

[1] Vol. vi, No. 24, p. 355.

[2] For his more complete statement and figures, see Flora, 1892, p. 92, &c.

[3] Morphologische und biologische Studien, Annales du Jard. Bot. de Buitenzorg, I. vii, p. 111.

[Annals of Botany, Vol. VII. No. XXVII. September, 1893.]

tective leaf of the male plant, and to its brown colour; the leaves of the female plant are also destitute of chlorophyll, and Haberlandt[1] has stated that 'assimilating foliage-leaves are entirely absent in the Buxbaumiae,' while he has further drawn attention to the apparent saprophytic habit of the rhizoids, their colourless thin membranes, and their frequent, mycelium-like anastomoses, as they traverse the humus in which they grow. Professor Goebel, in discussing these characters, points out truly that there is as yet no proof that *Buxbaumia* is actually saprophytic; but it would appear that, for the purposes of his argument, the burden of proof of this point will lie with him. Before conclusions can safely be drawn whether or not *Buxbaumia* is, as he suggests, 'an ancient type of moss which still retains a number of primitive characters,' it will be necessary to be more precisely informed on the point of its nutrition: if *Buxbaumia* really derives part of its nourishment as a saprophyte from the humus, there will be strong probability that the simplicity of its structure would be due to the reduction which usually follows such a habit. If, as Goebel suggests[2], the rotten tree-trunk acts only as a sponge, to hold water, and if the moss would grow equally well on a porous, inorganic substratum, such an observation would remove a serious objection to its being regarded as a primitive type: at present we are not informed on this point, and must, therefore, withhold a definite opinion. A further fact, mentioned and figured by Schimper[3], and noted also by Professor Goebel, appears to me to be very suggestive: he describes[4] how, though the young leaves of the female plant show no special peculiarities, the peripheral cells of the older leaves grow out into filaments with brown walls; some of these do not develop further, others grow into true protonema, while others, again, penetrating the soil, and elongating as rhizoids, 'convey nourishment to the plant.' The question is, *What* nourishment do they bring to this female plant, which,

[1] Pringsh., Jahrb. XVII, Heft. 3, p. 480, &c.
[2] Flora, 1892, p. 101.
[3] Bryol. Europ. vol. iv, suppl.
[4] Flora, 1892, p. 102.

though incapable of assimilation by its leaves, is still able to support the growing embryo of a relatively large sporogonium? It is possible, as Professor Goebel suggests, that the required organic supply is entirely derived from the assimilative activity of the protonema; but in presence of such a peculiar physiological condition as that above noted, I think that more exact proof of the mode of nourishment of *Buxbaumia* will be necessary before the facts relating to it can be accepted for purposes of morphological argument. I find it difficult to believe that we really see a primitive condition in a plant in which the leaves are incapable of assimilation themselves, though the plant receives its support indirectly through them, from outgrowths of a filamentous character, formed, comparatively late, from their margins. Whether the organic supply be exclusively derived from the assimilative activity of the green filaments or in part derived saprophytically from the substratum, the *indirectness* of the mode of supply, and the late appearance of the parts which supply it, are facts not easily reconciled with the suggested primitive character of the organism.

Nor does the comparison with *Diphyscium* appear to me to strengthen the case: the relations of these genera are not very close, though the similarity of their sporogonia is greater than that of their gametophytes. It is true the seta of *Diphyscium* is short (but I do not see that this is a fact of material weight), while that of *Buxbaumia*, the supposed more primitive form, is long. In *Diphyscium* the relatively large green leaves of the female plant have not the marginal protonema as in *Buxbaumia*: on this ground, as well as from the green colour of the leaves—that is, on grounds of *directness* of nourishment—*Diphyscium* would appear to me to be the more primitive form.

The relatively large bulk of the rhizoids to the bulk of the plants in both *B. aphylla* and *D. foliosum* is certainly striking; also the exceedingly fine, hypha-like ramifications into which they run, and their very complicated anastomoses, so as to form a plexus extending far into the substratum. I have also noted a frequent, though not constant, association of

fungal hyphae with the rhizoids of both genera: these are applied closely to the surface of their walls, even in specimens of *Diphyscium* brought freshly from the country. Such a juxtaposition may be merely accidental, though common: or the fungus may be simply parasitic, though I saw no perforation: or there may be a symbiotic relation between the organisms. I think such matters as these will have to be taken more fully into consideration before it can be admitted that *Buxbaumia* is really independent of external organic supply. It is not sufficient that Professor Goebel shall conclude, however justly, that 'we have as yet no proof of the saprophytism of *Buxbaumia*' (l.c. p. 101); before his view can be established, he must show that, notwithstanding the many suspicious points about its mode of life, *Buxbaumia* is really independent of saprophytic nourishment.

I would even go further, and remark that, if *Buxbaumia* were proved to be quite independent of an organic substratum as regards its nutrition, that would not at all prove its primitive character; for a saprophytic habit is only one of the factors which conduce to morphological reduction.

The line which Professor Goebel would draw between a plant which has stood still at an early stage of development (p. 102) and such as have undergone reduction is one of the most blurred lines in all morphology. I confess that, though some of the facts adduced by him appear to support his conclusion, still, in view of the facts above noted, it seems to me at present more probable that *Buxbaumia* is a reduced rather than a really primitive type of moss; and it would appear that the reduction has affected the vegetative organs of the moss-plant more than other parts.

Professor Goebel has also compared the simple gametophyte of *Buxbaumia* with that of *Trichomanes*: the similarity is certainly obvious enough, but the question will be whether we see in it anything more than an example of parallel development—that is, of comparatively recent adaptation—of one genus or of both, to somewhat similar circumstances. There is a close similarity of the conditions to which the

gametophytes of these two genera are exposed: they both grow in a humid atmosphere, while moist humus, commonly in the form of effete and decaying superficial tissues of tree-trunks, suits them both: neither the Hymenophyllaceae nor the Buxbaumiae can be considered entirely free from the charge of saprophytic nourishment (see Haberlandt, loc. cit. p. 482). I have elsewhere discussed this question of the similarity of the moss-protonema and the prothallus of *Trichomanes* at some length[1]: the main point is that the similarity depends on the *vegetative organs*, such as the filamentous protonema-like growth, and the small archegoniophore. But, though there is certainly some similarity of their antheridia, the archegonium of *Trichomanes* or of *Hymenophyllum* is a true fern-archegonium, as regards its segmentation and mature structure; and in point of its single neck-cell it is even less like a moss-archegonium than are those of certain other Vascular Cryptogams. The archegonium of *Buxbaumia* appears, however, to be a true moss-archegonium[2]. To me the dissimilarity of the archegonia of *Trichomanes* and of *Buxbaumia*, as regards form and segmentation, appears a more weighty fact than the similarity in vegetative conformation of the gametophyte, since the archegonium in ferns and mosses is relatively constant in its characters, while their vegetative conformation is not constant. When to this is added the suspicion of saprophytism, as well as the entire dissimilarity of the sporophyte in *Buxbaumia* and *Trichomanes*, the case against Professor Goebel's comparison appears to me to be a very strong one. In the light of the new facts contributed by Professor Goebel relating to *Buxbaumia*, I see no reason to alter the opinion set forth in my papers above quoted—viz. that such similarity of the gametophyte as is found in the mosses and Hymenophyllaceae, as regards their vegetative development, is probably the result of relatively recent adaptation, of one or

[1] Annals of Botany, vol. v. p. 109, &c.

[2] See Goebel's Figs. 12, 17, Pl. VIII, Flora, 1892; also Bruch, Bryologia Europaea, iv. Pl. I, Fig. 12.

of both, to similar external circumstances, rather than dependent upon primitive characters which they have had in common throughout their evolution.

I have elsewhere remarked [1] that the method of comparison of vegetative characters of the gametophyte, which Professor Goebel has adopted in treating plants so divergent in character as the mosses and ferns, is at variance with the methods commonly in use in the classification of phanerogamic plants. In these the conformation of the vegetative organs is usually treated as a secondary consideration, while the characters of the reproductive organs are given the precedence. Professor Goebel, however, appears to place the vegetative organs in the foreground of his argument, and attaches importance to their external form and structure, notwithstanding the entire dissimilarity of the sporophyte in the plants compared, and even the important difference of their archegonia. How cautious it is necessary to be in trusting to the vegetative conformation of the gametophyte in archegoniate plants is illustrated in the genus *Lycopodium*: here, without any marked difference of type of the sporophyte, the sexual plant varies within very wide limits of form, though the sexual organs remain essentially constant. The difference between the prothalli of *L. annotinum*, of *L. cernuum* and of *L. Phlegmaria* has been sufficiently demonstrated and remarked upon by M. Treub [2], while he specially points out the similarity of their sexual organs as regards structure and development. Such considerations make me doubt the wisdom of so far departing from the methods in general use among the higher plants as to press comparisons, based on similarity of vegetative conformation, in plants which show marked dissimilarity in other parts of such importance as the archegonia and the whole sporophyte generation. The fact that the organisms in question are lower in the scale does not appear to me a sufficient justification of this method.

[1] Annals of Botany, vol. v. p. 120.

[2] Ann. Jard. Bot. d. Buitenzorg, v. p. 88, &c.

In the same article[1] Professor Goebel offers certain criticisms upon my preliminary statement of results from the study of spore-producing members of the Vascular Cryptogams[2]. I would here remark that this was only a preliminary statement, and that readers are not yet in possession of the full facts or figures. In the meanwhile I shall endeavour to meet the most salient point of Professor Goebel's criticism.

While studying the evidence of sterilization of potential sporogenous tissue, I recognize fully the *correlation* which so often appears between spore-production and vegetative development. Upon this subject Professor Goebel has contributed very largely to our knowledge. The essential point on which we differ is the interpretation to be put upon this correlation. When Professor Goebel says (p. 359) that 'it can be experimentally proved that the sporophylls of Leptosporangiate Ferns are modified leaves'—that is, modified foliage-leaves—he makes an assumption in which I am unable to follow him. That there is a correlation between vegetative growth and spore-production he has satisfactorily demonstrated by experiment[3]; but I submit that his experiments do not touch the question of priority of origin of the sporophyll, or of the foliage-leaf, in point of view of descent. He appears to me to have assumed that the type of leaf which is prior in the ontogeny was also the first to appear in the phylogeny. Now this assumption was made by Goethe, though it was expressed in different terms, as was natural for a pre-evolutionary writer; by use it has become so familiar that those botanists of the present day who entertain some form of belief in evolution hardly recognize that, if they hold this opinion, it will be their duty to substantiate it. It seems hardly to have occurred to morphologists, even yet, that Goethe's views on progressive (fortschreitende) metamorphosis are incompatible with a belief in the descent of plants which show consistent antithetic alternation—that is, in which spore-production was throughout evolution a constantly recurring event.

[1] Annals of Botany vi. p. 358. [2] Proc. Roy. Soc. vol. l. p. 265.
[3] Ber. d. deutschen Bot. Ges. 1887.

In my view, *the progression from foliage-leaf to sporophyll, as seen in the development of the individual, cannot be assumed to illustrate the progression as regards descent.* The following considerations will explain this statement, which is made on the understanding that plants now living upon the earth illustrate, however imperfectly, the course which evolution probably took. From a comparison of these we learn that spore-production was the first office of the sporophyte, and that the spore-stage has recurred constantly in the life-cycle during descent. As the spore-production increased (the increase in numbers of spores being a manifest advantage) the powers of the gametophyte were insufficient to supply the necessary nutrition and external protection. The need for further supply appears to have led to the intercalation of a vegetative phase of the sporophyte, between fertilization and spore-production. In the Bryophyta the external protection and nutrition of the spores were supplied, but with only a minor degree of efficiency, by vegetative development of sterilized tissues of the lower and peripheral parts of the sporogonium; there is, however, no further elaboration of form beyond the occasional presence of chlorophyll, containing expansions of the apophysis. But in vascular plants the foliar development appeared: as to the details of the way in which it first arose we are still without definite information; much less do we know for certain whether the first leaves which appeared were sporophylls or foliage-leaves. When Professor Goebel writes, 'It can be experimentally proved that the sporophylls of the Leptosporangiate Ferns are modified leaves,' bringing this as an argument against me, he appears to me to assume, on ground of their priority in the ontogeny, that the foliage-leaves were of prior existence from the point of view of descent. I assert, on the other hand, that this is not proved, and that a good case could be made out for priority of the sporophyll; in which event the conclusion would need to be inverted—*the foliage-leaf would be looked upon as a sterilized sporophyll.* This would be perfectly consistent with the *correlation* demonstrated by Professor Goebel's

experiments, as also with the intercalation of a vegetative phase between the zygote and the production of spores.

But, for my own part, I should be diffident in making any general statement on the point of priority of the sporophyll or of the foliage-leaf from the point of view of descent, as applicable for all Vascular Plants; and it is not my present purpose to discuss this question at large. I desire now only to make it clear that there is an unproved assumption involved in the passage quoted from Professor Goebel's paper (p. 359), an assumption which, in the absence of proof to support it, appears to me to materially impair the validity of his argument.

If, however, it be contemplated as possible that, in certain cases, the foliage-leaf may be, in point of view of descent, a sterilized sporophyll, this would greatly alter the face of the discussion. I have already intimated [1] that in the Lycopods there is reason to believe that a sterilization of sporophylls has taken place, and that the result is to be seen in the foliage-leaves, which in most species differ from them in little beyond the absence (partial or complete) of the sporangium. To me, whether we take such simple cases as the Lycopods or the more complex case of the Filicineae, the sporangium is not a gift showered by a bountiful providence upon pre-existent foliage-leaves: the sporangium, like other parts, must be looked upon from the point of view of descent: its production in the individual or in the race may be deferred, owing to the intercalation of a vegetative phase, as above explained; while, in certain cases at least, we probably see in the foliage-leaves the result of sterilization of sporophylls. If this be so, much may then be said in favour of the view that the appearance of sporangia upon the later-formed leaves of the individual is a reversion to a more ancient type rather than a metamorphosis of a progressive order.

The acceptance of such views as those thus briefly sketched would materially alter the face of the discussion. While recognizing the *fact* of correlation as demonstrated by Goebel, and illustrated more or less clearly in so many spore-bearing

[1] Roy. Soc. Proc. vol. l. p. 270.

organisms, I hold that that fact, when stripped of any unproved assumption, is in accordance with such theoretical considerations as were put forward in the paper which Professor Goebel has criticized.

I would furthermore ask those who are disposed to disagree with me to bear in mind the opinions already expressed by me elsewhere[1] as to the probable relations of the Eusporangiate and Leptosporangiate Ferns. Though it is commonly held that the latter are the more primitive type, I have been led by careful consideration of the evidence to conclude that the preponderance of evidence is in favour of the view that the Eusporangiates are the more ancient forms: this question, however is still an open one, but the opinions stated in the paper quoted will necessarily affect the questions now under discussion.

Professor Goebel further remarks[2] that 'in *Ophioglossum palmatum* the sporophylls are still clearly recognizable as leaf-segments.' This view has also been entertained by one of my English critics, both using it as an argument against the theory that the 'fertile frond' of the Ophioglossaceae is an elaborated and partitioned sporangium, homologous with the smaller and non-partitioned sporangium of the Lycopods. I have carefully examined the numerous specimens in the herbarium at Kew, and write from previous knowledge of those in the British Museum: pending more detailed observations on alcohol-material, I find, from external examination, the following difficulties in the way of accepting the apparently simple view above quoted:—

(1) The arrangement of the 'fertile spikes,' when marginal, is indefinite, being neither regularly alternate, nor in pairs: this is, however, the usual arrangement for pinnae, including those of *Botrychium*.

(2) Many of the 'fertile spikes' are inserted *irregularly upon the adaxial surface of the frond*, not upon its margins.

(3) The 'fertile spikes' branch in very irregular fashion, there being apparently no common rule, though this is usually the case for pinnae.

[1] Annals of Botany, vol. v. p. 109, &c.

[2] Annals, loc. cit. p. 360.

By the term 'leaf-segment' or 'pinna' I understand a marginal lobe of a leaf, and pinnae are habitually arranged along the marginal lines, alternately or in pairs, and show a common rule of development. Occasional coalescence of *pairs* of such outgrowths across the adaxial face of the leaf are known. But in this case, if the 'fertile spikes' are pinnae or leaf-segments, comparable to those of *Aneimia* or of *Osmunda*, or the vegetative lobes of *Botrychium*, there must have been a frequent and irregular migration of *individual* pinnae from the margins to the surface of the frond: to such an irregular migration of individual pinnae I know no parallel. Quite apart from comparative considerations as brought forward elsewhere, this difficulty, together with the irregularity of distribution of the fertile spikes on the leaf, the frequency and irregularity of their branching, and the indefinite form of the terminal lobe, dispose me against this apparently simple explanation of the frond of *Ophioglossum palmatum*. I shall hope to have the opportunity of examining alcohol-material before stating that view of the nature of the frond in this species which I have entertained all through this work, but not yet stated, because want of alcohol-material had made a detailed examination hitherto impossible.

I wish also to reserve my answer to Professor Goebel's objections with regard to *Botrychium*[1]. I have long been aware of the frequent presence of sporangia on the usually sterile frond of *Botrychium*, and have had museum specimens showing it in my possession for many years: the fact of their presence is certainly a difficulty, which I shall hope to meet in the course of a general discussion of the subject.

The opinion appears to be held by some that the sporangium cannot undergo such elaboration of form and structure as I suggest. It is true that such elaboration has not hitherto been demonstrated, but those who are actively engaged in morphological inquiry will be disposed to believe all things possible, though all may not be convenient. The fact that demonstration has not yet been given does not preclude its

[1] loc. cit. p. 359.

possibility, and certainly does not justify the statement that it cannot occur.

The suggestions which I have offered in my studies on spore-bearing members involve the thesis that there are potentialities of elaboration of such parts as we style sporangia, analogous to the potentialities of axis, leaf, root or hair. Every one of these parts is susceptible of variations in size, form, and structure, in different plants, while all may be branched: in certain species, genera, or even families, the branching of any of these parts may, it is true, be in abeyance; but speaking of these parts generally, the potentiality of branching is one of their characters. What good reason is there for assuming that sporangia have not this potentiality, and thus differ from all other parts of the sporophyte?

In point of structure great fluctuations are seen in axes and leaves, or their parts: vascular tissues, habitually present, may be entirely absent in certain cases, while the morphological character of the part is not considered to be thereby affected. Conversely, emergences, which are commonly without vascular supply, are in some cases traversed by vascular bundles; but they are not by reason of this elevated to a higher morphological category. Accordingly, the presence or absence of vascular bundles in a given part need not affect our view of its sporangial character: it is to be noted that though vascular tissue is usually absent from sporangia, it is present in the ovules of Phanerogams, which are generally admitted to be sporangia.

With regard to branching also a similar line of argument may be used, and it may be pointed out as against the fact that branching of sporangia is not generally recognized, that branched sporangia do occur in *Salvinia natans*: while the megasporangia of this species are attached by separate stalks, the microsporangia, otherwise similar as regards position, are borne on repeatedly branched pedicels. I have not yet worked out the details of development of this interesting case, but a good representation of an advanced stage is given in Rabenhorst's Kryptogamen Flora, vol. iii. p. 603.

Thus, if we apply consistently to sporangia the same morphological methods as to axes or leaves, we shall be prepared to recognize in them the possible presence of vascular tissue, and the potentiality of branching; and in my opinion we should not be justified in excluding a part from the category of sporangia because it shows evidence of branching, or of vascular tissue, or even of both together.

A further factor necessary for my views is the partitioning of the sporangium: on this point it is hardly necessary to remind readers that partial sterilization of a potential archesporium has been recognized in the Bryophyta, in *Isoetes*, and in the ovules of various Phanerogams. In particular, Professor Goebel has shown that the trabeculae of *Isoetes* are produced from sterilized archesporial tissue, and he allows that they serve a useful purpose: but he remarks[1] that he would attach only a biological, that is, an adaptive significance to the fact that they are the result of partial sterilization of the sporogenous tissue, and that it is a question how far sterilization may proceed in sporangia and sporophylls. I agree with him that this is a question open for discussion; but it appears to me that if sterilized portions of a potential sporogenous tissue are admitted to serve a useful purpose, and if such occur in forms which we believe to be relatively low in the scale of plants, there is a reasonable probability that such sterilization will have played a part in the evolution of other forms, and it will be our duty to see whether traces of similar sterilization occur among other early types. Synangia are a marked feature in certain Vascular Cryptogams: they have commonly been looked upon as the result of coalescence of sporangia originally distinct; but I submit that it is a possible view that they may have been derived by a partial sterilization and formation of partitions in originally simple sporangia[2]. It is on grounds of detail of development and comparison of plants akin to one another that this question can best be solved: it would be leading us beyond the limits of such an article as this to enter now upon

[1] loc. cit. p. 358.

[2] See Annals of Botany, v. p. 131.

a detailed comparison: reference only will be made to communications relating to the Lycopodinae and Psilotaceae which have been laid before the Royal Society[1]. In these I have brought forward developmental and comparative evidence supporting the view that the synangium of the Psilotaceae is the result of septation of a Lycopodinous type of sporangium, while *Isoetes* and *Lepidodendron* provide some interesting intermediate features. If this view be correct, we should there see examples not only of partial sterilization, but of formation of complete septa from the sterile tissue: that is of partitioning of the sporangium. This is the third factor above alluded to. If this factor occur simultaneously with the other two (viz. branching and formation of vascular tissue, of which examples have been above quoted, and of which incipient steps may be seen in the Psilotaceae), then the result might very well be such elaboration as I have suggested that we see illustrated in the 'fertile frond' of the Ophioglossaceae.

The rather extensive area of facts included in the papers above cited will have to be considered by those who are in doubt as to my conclusions, and still more by those who appear disposed to take an attitude of negation. My conclusions as to the part played by spore-bearing members in the evolution of the sporophyte may be wrong, and are probably susceptible of amendment: my present object has been to show that they are not to be disproved off-hand. I have no wish to avoid criticism, but I would venture to point out to my critics that they are not yet in full possession of the facts, or of the reasoning to be based upon them. Till a full statement is published, criticism must be more or less premature; it may be accepted as 'preliminary criticism,' in the same way as the statements criticized were 'preliminary statements.'

[1] Roy. Soc. Proceedings, vol. l. p. 265; vol. liii. p. 19. Also abstract of the detailed memoir, part i. read June 15, 1893. The conclusion of my paper on *Lepidostrobus Brownii* (see above, p. 351, &c. of this volume of the Annals) should also be consulted.

NOTES.

SYNANTHY IN BELLIS.—In July of last year a gentleman in the north of England sent me a specimen of a 'new British plant.' The specimen did not arrive in very good condition, and the appearances it presented were so peculiar that it was no matter for surprise that the plant was not at first recognized as the common Daisy. Further examination, however, rendered it certain that the plant was none other than *Bellis perennis*. A note was taken of the structure of the flower, but as the sender was good enough to send also some plants, I decided to await the production of other flowers before publishing any account of their peculiarities. Some of the plants were grown throughout the winter by my friend Mr. Worthington Smith, in Bedfordshire, and others were grown by myself in Middlesex. This year both sets of plants have produced their flowers, and substantially of the same character as those formed last autumn in Northumberland. The flowers have been analysed both by Mr. Smith and myself, and Mr. Smith has kindly furnished me with the sketches which accompany this note.

The changes observed are first the production on the scape of a detached leafy bract with a distinct petiole and a narrow blade. This bract may be taken to be intermediate between the ordinary foliage-leaves and the bracts of the involucre. The young flower-head has an oblong rather than a spheroidal form; the bracts of the involucre are fewer in number (nine in two rows), and less widely spreading than customary. The ray-florets are of the usual colour, but much less numerous than is generally the case (only five)—some are spreading, others erect and more or less twisted, and enclosing a two-lobed style as usual. The florets of the disc are represented, not by separate corollas in great numbers, but by a single petatoid cup composed of several corollas, apparently flattened out as in the ligulate florets and united margin to margin. The free border of the tube shows lobes and other indications of its composite nature. Within this cup are the stamens, very numerous, completely detached and in a single row. The anthers are linear, apiculate, and longer than the filaments.

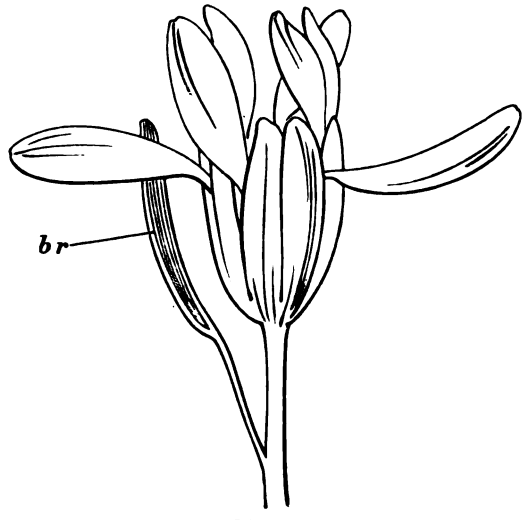


Fig. 1.

Fig. 3.

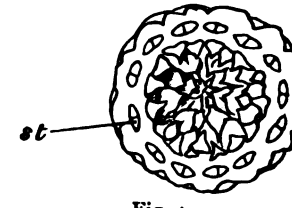


Fig. 4.

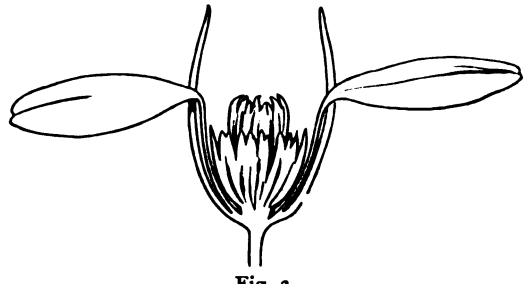


Fig. 2.

Fig. 5.

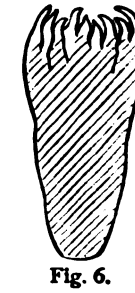


Fig. 6.

WOODCUT 1.

Fig. 1. Flower-head, showing partially detached involucral bract *br*, the other bracts ascending; ligulate florets few in number, some spreading, others erect. ×4.

Fig. 2. Longitudinal median section through flower-head, showing two involucral bracts, two ligulate florets, a cup consisting of conjoined tubular florets and a central club-shaped mass. ×4.

Fig. 3. Cup formed of conjoined corollas, detached. ×8.

Fig. 4. Transverse section (plan) showing the conjoined corollas, the stamens and the central axis. ×8.

Fig. 5 shows one of the hypogynous stamens and the central club-shaped axis. ×8.

Fig. 6. Longitudinal median section of central axis. (Drawn by Mr. W. G. Smith.)

The stamens surround a club-shaped dilatation of the axis which occupies the centre of the flower-head. It is solid and undivided below, but above gives off a number of deltoid processes which doubtless represent bracts or paleae. In no case is there any trace of styles, ovary, or ovule, except in the ray-floret.

How all these changes are brought about can only be certainly determined by following the course of evolution. The appearances lead to the inferences that there have been alternate accessions and arrests of growth, or even that the force of development was disproportionately great in one part, while it was, at the same time, small in another. By some such means the concrescence of numerous corollas into one composite tube, the detachment or lack of union of the stamens, and the lengthening and dilatation of the axis may have occurred.

I have never observed anything like this malformation in Compositae. The nearest approximation to it that occurs to me is that very common malformation in the Foxglove, *Digitalis purpurea*, in which the corollas at the upper part of the raceme are blended into one terminal cup. In these cases the axis is generally prolonged and thickened, and the result is often the formation of a flower so like in form to that of some *Campanula*, that I occasionally receive specimens with the information that they are the result of cross-fertilisation between a *Campanula* and a *Digitalis*! A similar case in *Myosotis*, but even more complicated, is described by myself in the Gardeners' Chronicle, August 8, 1891, p. 159, with a figure, and is the subject of comment by Professor G. Henslow in the Journal of the Royal Horticultural Society, vol. 15, August, 1893, p. xxvii.

MAXWELL T. MASTERS, London.

CHANGES IN THE RESERVE MATERIALS OF WHEAT ON KEEPING.—A sample of wheat which had been stacked for nearly thirty years, at Wingham near Canterbury, and recently thrashed, was given to me by the senior Bursar of St. John's College last March.

I made a complete analysis of this sample, the result of which seems to me interesting from a physiological standpoint, and is given in full compared with an analysis by exactly similar methods of a new sample grown last year on the same ground.

It is obvious that the two cases compared are not exactly parallel:

but a comparison with the analysis of a sample from the same ground shows more than a comparison with a general typical analysis of a fresh wheat, and I delayed the publication of the original analysis until that of the new sample, which the Bursar kindly obtained for me from Mr. Petley, was completed.

In the old sample the insoluble plastic compounds—proteids, starch, &c.—have undergone a considerable change in the direction of producing substances soluble in water, although the sample is now much drier than a normal wheat.

These changes suggest ferment-action and are probably due to hydrolysis. In the case of the proteid changes it is not certain that the alteration can be attributed to hydrolysis, as we do not know the exact relationship in which the soluble (legumin-casein) proteids and peptones stand to the proteids (gluten-fibrin) insoluble in water but soluble in dilute alkali. The increase in the amount of dextrin and reducing sugars is clearly a case of hydrolysis, which may have been caused by a slow action of diastatic ferments, although there are now no traces of these or of proteolytic ferments in the old sample as shown by the analysis. It seems probable that such ferments were originally present and produced the changes during the earlier period of keeping, but have since been destroyed either by oxidation or the influence of micro-organisms. Diastase in a solid state is particularly liable to such attacks, and it is not impossible that the whole of the alterations in question may have been produced by micro-organisms.

It may be added that the old sample is apparently dead, as after careful soaking for various periods none of it has as yet shown any signs of germinating, although it has been kept under favourable conditions for more than two months. The old sample rapidly becomes covered with 'mould' when placed on sand after soaking, but a microscopic examination has not shown the presence of any spores in the tissues or other abnormal appearance beyond what might be expected from the somewhat unusual dryness of the grains.

The 'gluten' was not examined with an aleurometer, but it has the characteristic properties of ordinary wheat 'gluten' and gives a very deep yellow coloration with nitric acid.

As there is 14 per cent. of water in the new samples against 9 per cent. in old, the figures given below do not exactly represent the ratio of any constituent to the total dry weight in the same way, but this can easily be calculated for any constituent from the values given.

The note received with the old wheat contains the following particulars:—

Stacked at Wingham in 1864; thrashed in 1892. Stacked in field on which grown; the whole crop of a field about twenty acres. Thatched in ordinary way with straw; thatch repaired at intervals.

The new wheat was a portion of last year's (1892), crop, stated to have been got in under rather unfavourable circumstances, and not in very good condition; but 90 per cent. of it germinated readily when tested.

	Old wheat, stacked for 28 years		New wheat, from same ground	
Water		9·0		14·0
Nitrogenous compounds:—				
Gluten-fibrin proteids (insoluble in water, soluble in 2 p. c. Na OH.)	4·3		7·1	
Legumin-casein proteids (soluble in water, precipitated by cupric acetate)	3·6		0·8	
Peptones	0·3	11·5	0·4	10·2
Amides (calculated as asparagin)	0·8		nil	
Nitrogenous compounds (other than proteids, peptones and amides)	2·5		1·9	
Starch		60·0		68·0
Dextrins		6·0		1·1
Sugars		6·2		nil
Fat		1·6		2·0
Cellulose &c. (residue insoluble in dilute acid and alkali)		3·5		3·0
Ash		1·7		1·5
		99·5		99·8
Diastatic power (on starch-solution) of finely-divided substance suspended in water		nil		considerable
Proteolytic power (on gluten-fibrin proteid) of finely-divided substance suspended in water		nil		considerable

The determinations of water, starch, dextrins, sugars, fat, cellulose, &c., ash, were made by the ordinary methods used for analysis of cereal grains.

The determinations of the nitrogenous constituents were made as follows:—

Total nitrogenous compounds by extracting with 2 per cent. NaOH, evaporating solution to dryness, and estimating total nitrogen by Kjeldahl's process.

In the more detailed analysis of nitrogenous constituents a portion of the material was first extracted with cold water and the residue then again extracted with 2 per cent. NaOH.

The gluten-fibrin proteids were determined in the NaOH solution, as in the estimation of total nitrogen by Kjeldahl's process.

The legumin-casein proteids were determined in the cold water solution by precipitating with copper acetate and estimating nitrogen in precipitate by Kjeldahl's process: the peptones in filtrate from above (after removing the copper with H_2S), by precipitating with sodium phosphotungstate, after addition of dilute sulphuric acid, decomposing the precipitate with alkali, and estimating nitrogen in a portion of alkali solution by Wanklyn's albuminoid ammonia process; distilling with alkaline potassium permanganate and 'nesslerising' ammonia in distillate: the amides in filtrate from above precipitate by boiling with dilute acid and determining (combined) ammonia produced by sodium hypobromite in nitrometer, calculating results as asparagin.

By subtracting the nitrogen as proteids, peptones, and amides from the total nitrogen and multiplying by 6·33, the results entered as nitrogenous compounds, other than proteids, &c. were obtained.

To determine the diastatic power, 10 grains of finely-divided substance was suspended in water to which a little chloroform was added and allowed to act for four hours on 1 per cent. starch-solution at 25°, testing at intervals by iodine-reaction.

The proteolytic power was examined in same way, substituting for starch thoroughly washed gluten (obtained by neutralizing an NaOH extract exactly with dilute acid) suspended in water, and testing whether there was much increase in the precipitate caused by copper acetate at end of twelve hours, compared with the precipitate given by another 10 grains of same sample suspended in pure water only, after filtering through paper without heating in both cases.

The sugar, in the old sample, was chiefly maltose, as the solution (after separation of the dextrins) in which it was determined gave very little reduction of copper acetate solution acidified with acetic acid, and therefore contained only traces of glucose.

Very little accurate information has been recorded with reference to the changes which may take place in the reserve materials of seeds during prolonged keeping under conditions unfavourable to germination, and it seems to me desirable that a careful analysis should be made in a number of such cases when opportunities occur of obtaining suitable material.

E. HAMILTON ACTON, Cambridge.

St. John's College Laboratory,
June 10, 1893.

THE ALEURONE-LAYER OF THE SEED OF GRASSES.—The various botanists who have investigated the seeds of grasses, have made conflicting statements as to the precise nature of the contents of the cells composing the so-called aleurone-layer. For this reason, and on the strength of some unpublished observations of his own, Professor Vines suggested to me a re-investigation of the subject, at the same time putting forward the hypothesis that the cells in question chiefly stored up phosphates.

Not to enter too deeply into the history of the subject, it may be said that the aleurone-layer was, for a long time, regarded as simply and solely a reservoir for proteids.

In 1872, S. L. Schenk[1] published some observations of the contents of the aleurone-layer of the wheat-seed. He stated that the grains, which formed the chief portion of the contents of the cells, did not consist of proteids. He found that these grains gave no proteid reactions; were insoluble in ether and alcohol, in water, and in dilute hydrochloric acid; and that they were not attacked by proteolytic ferments.

Later Johanssen[2] pointed out that fatty oil was abundant in the cells, as also proteid grains which were easily soluble in water.

Lüdtke[3] stated that the cells contained protoplasm, oil, and aleurone-grains. He said that the aleurone-grains were insoluble in water or in dilute caustic potash, and that they contained no globoids or other enclosures.

[1] S. L. Schenk, Anatomisch-physiologische Untersuchungen, pp. 32–37. Wien, 1872.

[2] W. Johanssen, Sur la gluten et sa presence dans le grain de blé. Résumé du Compte-rendu des travaux du laboratoire de Carlsberg. 2me vol. 5me livr. 1888.

[3] Lüdtke, Beiträge zur Kenntniss der Aleuronkörner, Pringsh. Jahrb. xxi. 1889.

Text-books make no mention of globoids in these aleurone-grains of grass-seeds.

Haberlandt[1] casually figures globoids as occurring in the aleurone-grains in this layer in the seed of the oat, whereas Brown and Morris[2] neither mention nor figure globoids in the aleurone-grains of these cells in the barley-seed.

The present investigations lead to the result that the cells composing the aleurone-layer contain a rich stock of oil in the protoplasmic network, whilst in the meshes of the protoplasm are numerous aleurone-grains which form the greatest portion of the cell-contents. But in most cases the aleurone-grains consist chiefly of globoids with only envelopes of proteid matter. In such a case a typical aleurone-grain is roughly spherical, and comprises a small proteid shell encasing a large central globoid. Hence it is often the case that the aleurone-layer is notably a receptacle for phosphates, as Professor Vines suggested. As regards the function of the layer I made no observations. The following methods were adopted and in typical cases gave the following results.

(i) Sections, cut from *dry seeds*, were placed in 50 *per cent. solution of alkannin*, and after a time were mounted in dilute glycerine. The whole aleurone-layer was stained red. The cells were seen to contain red globules, or even a red network in the meshes of which lay unstained grains. Other tests for oil confirmed these results[3].

(ii) Sections were cut by the aid of a razor moistened with absolute alcohol. They were then dropped straight into *absolute alcohol*, and left for periods varying from a few seconds to some hours. In most cases, however, only a short time elapsed before they were removed. They were then transferred into *benzole, turpentine, xylol, or a mixture of ether and absolute alcohol.* The oil was thus removed, and the sections were once more conveyed into *absolute alcohol.*

Examined at this stage the cells appear filled, in a typical case, with many minute ring-like bodies (really spheres)—the aleurone-grains—embedded in a network of protoplasm.

These sections were then treated in three different manners.

(*a*) Stained with *iodine* or *proteid-staining dyes*, the protoplasm

[1] Haberlandt; Ber. d. deutsch. bot. Ges., vol. 8.

[2] Horace Brown and Morris; Journ. Chem. Soc., 1890.

[3] Black colour with osmic acid; insolubility in sulphuric acid; solubility in ether and alcohol, turpentine, &c.

and the peripheral parts of the aleurone-grains become coloured, but the central parts of the grains remain colourless.

(*b*) Treated on the slide with a 1 *per cent. solution of caustic potash*, the protoplasm swells up and partially emerges from those cells which have been cut open: the peripheral parts of the aleurone-grains dissolve, but the central parts of the grains remain unchanged, so that at this stage the cell-contents present the appearance of a hyaline mass in which are suspended innumerable spherules. After washing thoroughly with water, the spherules dissolve in 1 *per cent. acetic acid* or in *picric acid*. Hence they are not composed of calcic oxalate, or of proteids, but are probably ordinary globoids. This is confirmed by their dissolving in an *ammoniacal mixture of ammonic chloride and hydrodisodic phosphate*: here they are replaced by well-defined crystals of double phosphate of magnesium and ammonium (PO_4MgONH_4). This proves that the spherules contain magnesium. Heated dry on a coverslip, after the washing away of the caustic potash, the spherules turn brown but retain their shape even after prolonged heating—thus showing that the spherules are globoids.

(*c*) Treating sections which have been freed from oil with *picric acid*, and washing well with absolute alcohol, the spherules disappear from the aleurone-grains which now remain behind as hollow spheres. Treating such preparations with a 1 *per cent. solution of caustic potash*, no spherules remain behind. If *ammoniacal ammonic chloride and hydrodisodic phosphate* be supplied to the sections there is no deposit of crystals. Hence the spherules have been dissolved by the picric acid, and it is they which contain the magnesium.

(iii) To avoid any possibility of error due to the use of absolute alcohol, the above-described methods were applied to sections from which oil had not been removed and which had not been treated with alcohol. These observations confirmed the previous ones, though the presence of oil made it much more difficult to see the nature of the aleurone-grains.

As will be seen in the special work described later on, the aleurone-grains in the cells in question sometimes contain no globoids.

As to the nature of the proteids in the aleurone-grains, I devoted but scant attention to the matter. But after the employment of alcohol the proteids of the aleurone-grains are at any rate partially insoluble in water. Under the same circumstances, in *Zea Mays*, they are not dissolved by 1 per cent. caustic potash, even after

twenty-four hours. Professor Vines informs me that similarly insoluble proteids occur in the form of aleurone-grains in the seeds of *Musa Hillii*. But I have no doubt that Lüdtke and Schenk, in their work, mistook the globoids for the intact aleurone-grains. Hence their statements of the insolubility of these grains of the aleurone-layer by peptic ferments and in dilute acids (Schenk), or in caustic potash (Lüdtke).

By means of identical methods I worked out the distribution of globoids in the embryo of the seeds investigated.

Special Work.

Coix Lachryma.—The aleurone-layer is but one cell thick. Each aleurone-grain consists of a shell of proteid matter enclosing a single central globoid. Oil occurs throughout the whole of the embryo, and in the aleurone-layer, but not elsewhere in the endosperm. Globoids are found abundantly in the embryo and in the aleurone-layer [1].

If the microchemical methods adopted be correct, we should expect a chemical analysis to show that the embryo is rich in phosphates, in magnesium, and calcium—corresponding to the vast number of globoids present in most of the cells composing the embryo. We should anticipate that there would be less ash in the endosperm—corresponding to the fact that it is mainly composed of starch, with but little proteid matter, and with globoids only occurring in the outermost layer. We should expect that this ash would contain phosphates, magnesium, and calcium, in considerable quantities: but that the calcium would be present in relatively larger quantities in connexion with the great amount of carbohydrate material, quite apart from the globoids.

And analysis reveals the fact that our anticipations are correct. For this analysis I am indebted to Mr. J. J. Manley, of the Chemical Laboratory, Magdalen College, Oxford. It will be seen that no account is taken of the potassium present.

	Endosperm.	Embryo.
Water and organic matter .	99·4954%	88·7151%
Ash	·5046	11·2849
	100	100

[1] In neither this seed nor in any other could I establish the presence of globoids in the epithelial layer of the embryo. But in *Coix* minute granules occur in the protoplasm of the epithelium; and these appear to answer the tests for globoids as far as their minute size enabled me to judge.

	Endosperm.	Embryo.
Water and organic matter .	99·4954	88·7151
SiO_2 &c.	·0134	·0409
Fe_2O_3	·0076	·0514
CaO	·0153	·1022
MgO	·0545	2·2139
P_2O_5	·4140	7·7520
Undetermined constituents .	....	1·1245
	100·0002	100·

	Endosperm.	Embryo.
SiO_2	2·6565	·3623
Fe_2O_3	1·5087	·4561
CaO	3·0360	·9058
MgO	10·8043	19·6190
P_2O_5	82·0540	68·6942
Undetermined constituents .	....	9·9626
	100·0595	100·

Oats, Rice, Rye, Wheat.—The aleurone-layer is single. The aleurone-grains contain globoids both in this layer and in the cells of the embryo. In the embryo of the oat the distribution of the globoids may be taken as typical, and is well marked. The globoids are most numerous in the scutellum and in the periblem of the root: they are less abundant in the plerome of the latter, and absent from its epidermis. Oil occurs throughout the whole of the embryo and in the aleurone-layer in all four types.

In the rice-seed minute starch-grains are found in the same cells as those in which aleurone-grains are so abundant—a somewhat exceptional circumstance.

Barley.—The aleurone-layer is not single, but for the most part several cells in thickness. In it each aleurone-grain has an envelope of proteid surrounding a single central globoid. In the embryo the globoids are small but very numerous. Their distribution in the root is sharply defined. The outermost three layers contain no globoids; the four succeeding layers are rich in them; whilst the whole centre of the root is devoid of them.

Sorghum vulgare.—The aleurone-layer of the seed consists of a single layer of flattened cells. In them there is protoplasm, oil, minute starch-grains, and aleurone-grains which, however, have *no globoids*. No crystals are formed in these cells when they have been treated with ammoniacal ammonic chloride and hydrodisodic phosphate. In the cells of the embryo occur fat, much starch, and aleurone-grains with large globoids. The distribution of the starch-grains corresponds with that of the globoids. Hence in this seed iodine-reagents have to be employed after the usual tests for globoids have been performed.

Zea Mays.—The aleurone-layer is single. In it the aleurone-grains contain small globoids, but have a larger proportion of proteids than in any of the preceding types in which globoids are present. When sections are placed in ammoniacal ammonic chloride and hydrodisodic phosphate, a small quantity of crystals form in the aleurone-layer. If placed in sulphuric acid, crystals of calcic sulphate form in these cells. After treatment with alcohol the proteids are insoluble in dilute potassic hydrate (1 per cent.). The embryo contains oil, and aleurone-grains with globoids.

PERCY GROOM, Oxford.

ON NUCLEAR DIVISION IN THE POLLEN-MOTHER-CELLS OF LILIUM MARTAGON.—Having been for some time past engaged in researches upon the mutual relations of the cytoplasm and nucleus, during spore-formation, in certain of the lower plants, I became desirous of working through some well-known type of division in the corresponding cells of a phanerogam, in the hope that some of the difficulties which presented themselves, during the investigations referred to, might thereby be explained.

For this purpose I selected the anthers of *Lilium Martagon*, in which the changes which accompany the development of the pollen grains had already been studied by Guignard[1]. He found, and my experience also confirms this, that alcohol is on the whole the best fixing and preserving medium for plant-cells, and my observations soon became chiefly directed to material which had thus been fixed.

I made use extensively of the various and numerous stains commonly associated with researches of this nature, both with and without the additional employment of mordants, but after a large number of

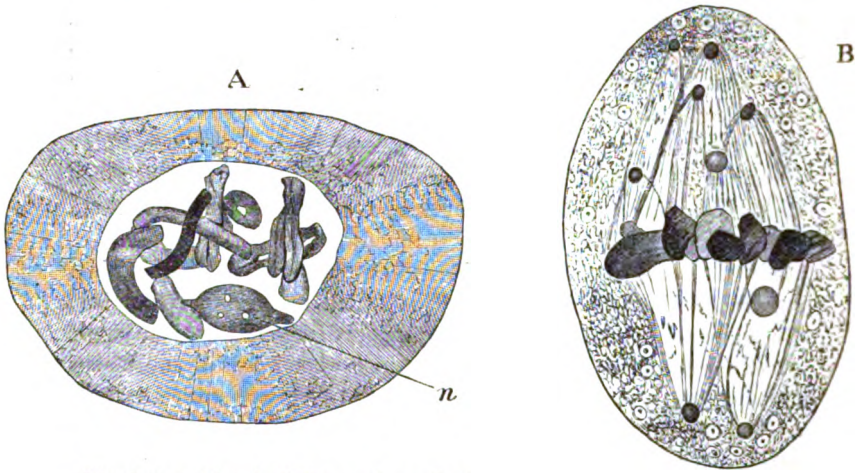
[1] L. Guignard, Nouvelles études sur la Fécondation, An. sci. nat. Bot., sér. 7, t. 14, 1891.

experiments, I came chiefly to rely on safranin, haematoxylin, fuchsin, methyl-green, gentian-violet and orange-G. On the whole, the best results were obtained by slow staining in haematoxylin, followed, after washing out the surplus stain, by further treatment with a watery solution of orange. Both gentian-violet and safranin, especially when used in conjunction with orange, gave good results. The sections thus prepared were mounted in glycerine, or glycerine and chloral hydrate, or were cleared and mounted in Canada balsam, and for double or triple stained preparations I much prefer the last mentioned mounting-medium. Extremely satisfactory results were obtained, in the case of the haematoxylin-orange stain, by treating the sections stained with haematoxylin, after careful washing, with a weak solution of lead acetate, and then finally, after again washing, staining with orange. Zinc sulphate was also tried in the same way, but was found not to give such good results. All the above methods led to the same conclusions, though with various degrees of clearness, and as my observations are somewhat out of accord with those commonly accepted, I thought it best to communicate those results which appeared to be of the greatest interest, reserving a detailed account for a future occasion.

On examining the pollen-mother-cells at that stage of division when the chromosomes (which, as Guignard stated, are twelve in number) are aggregated in the equatorial plane, and the achromatic spindle is well defined in the cytoplasm, I found what I was by no means prepared for, namely, that in the cytoplasm there are scattered about a number of *granules*, which were not figured in the plates accompanying the memoir already referred to; and that these granules are coloured by those stains which differentiate the chromatic elements of the nucleus, and are thus very clearly defined in the cell-protoplasm. They occur for the most part, though by no means entirely, in the region of the achromatic spindle, and the point of interest connected with them is this, that many of them are obviously related to the spindle-fibres, and mark the position of attraction-centres for parts of the spindle which is thus *broken up and becomes multipolar*, if one may use such an expression. This character is illustrated by the figure (Woodcut 2, B), which is intended to reproduce an actual *camera lucida* drawing. The granules which are thus related to the spindle-fibres are very variable in number, and are equally so in position; a number of granules, however, so far as I have observed,

always remain neutral. Those, however, which are so related to the fibres, exert a most obvious influence on the direction of these, and a whole sheaf or strand of spindle-fibres may sometimes be seen to divert from the main direction, and to end blindly on such granules.

I have been unable to demonstrate the existence of special centrosomes occupying definite positions at each end of the cell, and I am not clear as to how they might fit in with the granules. Perhaps it is conceivable that in some cases the individual granules might fuse and form one large terminal body which one might then regard as a 'centrosome,' and it is certain that in many cells the main portion of the



Woodcut 2—Fig. A. Pollen-mother cell of ***Lilium Martagon*** in early stage of division : *n* the nucleolus. B. Later stage, showing multipolar spindle.

achromatic spindle does stand in relation to bodies near the poles of the cell, but even in these cases there are almost always to be seen, in preparations which allow of a definite conclusion being arrived at, divergent strands separating from the main spindle and terminating in granules otherwise situated. Eventually, during the separation of the daughter-chromosomes, the spindle seems to become more regular, but the difficulties of tracing its real relationships simultaneously increase, and I do not as yet feel able to speak with entire confidence as to its further fate.

Shortly after I had, somewhat unwillingly, arrived at the conclusion

that these granules, with their curious relations to the spindle-fibres, were regular and normal constituents of the cell during these stages of division, I learnt with pleasure from my friend, Mr. J. E. S. Moore, that in the course of his investigations into the behaviour of dividing cells in animals, he had come across a similar case of multipolar spindles in *Branchippus*. His results are in course of publication, but he kindly invited me to see his preparations, and they agreed with those of *Lilium* in their essential characters so far as the relations of the spindle are concerned. Of course multipolar spindles in plants are not new, but those hitherto described[1] are of a different nature from those which occur in the Lily anthers. Moreover they are found in endosperm cells, whose nuclear constitution appears to vary within considerable limits, at least judging from the existing accounts before us. In such a cell-division as that which results in the production of a spore, it seems, however, possible that their occurrence may be of some special significance, but I reserve the discussion of this point for a future occasion.

Hitherto I have only described the cells when in a state of active division, but when these stages are compared with earlier ones, several other features of interest become prominent. The cytoplasm up to the time immediately preceding the disappearance of the nuclear membrane, and the aggregation of the chromosomes in the equatorial plane, is *perfectly free from stainable granules*. The nucleus, when preparing for division, exhibits a much convoluted thread with rows of dots, which stain deeply, running along the edges, exactly as is beautifully shown in Fig. 10, accompanying Guignard's memoir already referred to. The nucleolus is of enormous size, and contains usually several endonucleoli. At a stage somewhat later, when the chromosomes have become individualized, and are lying irregularly disposed within the nucleus, the nucleolus almost always assumes a most curious and characteristic shape, that of an oval body with two polar protuberances (Fig. *A*). Frequently it lies close to the cytoplasm, one of its pointed ends actually appears to jut out into it; sometimes, however, instead of one large nucleolus, stages in its fragmentation may be observed, and a number of smaller bodies, which present precisely the same staining-capacity take its place. Their aggregate size, however, is equivalent to that of the large single nucleolus.

[1] Cf. Strasburger, Histologische Beiträge, Heft 1, 1888, especially Taf. III, Fig. 34, and the text.

Immediately following this stage in the nuclear division, the chromosomes congregate in the equatorial plane, and simultaneously the granules already referred to in the earlier part of this communication are discoverable in the surrounding cytoplasm. They stain precisely as do the granules which arise in the nucleus by fragmentation of the nucleolus, and are very distinctly seen, many of them influencing the direction of the spindle-fibres as already described. It would at present be premature to attempt to do more than suggest that there may be a closer connexion between the granules of nucleolar origin and those which later occur in the cytoplasm, but it may be mentioned that Hertwig[1] suggested the possibility of a nuclear origin for the animal centrosomes. There are certainly great difficulties in the way of accounting for the sudden and abundant formation of granules on the assumption of a purely cytoplasmic origin, and their obvious relation to the spindle-fibres, as well as their ultimate fate, opens out a whole set of further questions. I will not advert to them at greater length here; I hope to be shortly able to speak more definitely on these, as well as other points raised in this preliminary note.

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THE GENUS TREMATOCARPUS[2].—At Mr. Hemsley's request I have examined the structure of the fruit of *Lobelia macrostachys*. Dr. Zahlbruckner's note on his *Trematocarpus macrostachys* may be divided into two portions, the first of which is decisive for the second, viz. the structure of the fruit, particularly with regard to dissemination, and the generic value of the characters derived from that particular structure. The remarks which I have to make on this structure are based upon observations on the material preserved in the Kew Herbarium, and on a fruit sent by Dr. Zahlbruckner to Mr. Hemsley.

The ovary wall of *Lobelia macrostachys*, Hook. et Arn., exhibits the same general structure as in allied species of *Lobelia*, the generic identity of which has never been doubted. One remarkable character is the presence of a well-developed system of vascular bundles anastomosing in a distinct network very like that found, for instance, in *L. nicotianaefolia*, an Indian species, with the only difference that it is

[1] O. Hertwig, Die Zelle und die Gewebe, Jena, 1892, p. 48 and 164.
[2] See Annals of Botany, vol. vii. No. 26, and vol. vi. No. 21.

of a stronger texture. When the fruit approaches the mature state, the inner part of the pericarp of these species begins to transform into a thin endocarp of a papery or cartilaginous nature, whilst the remainder dries up gradually. But whereas it hardly undergoes any particular transformation during that state in *L. nicotianaefolia* or in *L. hypoleuca*, a Hawaian species, the network of vascular bundles soon becomes very prominent in *L. macrostachys* and assumes the character of mechanical tissue which is formed by the elongated cells surrounding the vascular bundles becoming sclerenchymatous. These sclerenchymatous masses are often confluent forming broader bundles, and they anastomose according to the disposition of the vascular bundles, thus forming a net-like woody skeleton. This skeleton immediately overlies the endocarp and is generally quite distinct, even before the latter has assumed its cartilaginous character. It is enclosed by a thick parenchymatous epicarp with a delicate epidermis. The epicarp dries up and contracts, but as the inner skeleton and the external ribs of the capsule are much stronger, it must break up itself. It becomes distinctly thinner over the interstices of the framework over which it is expanded. Minute cracks and holes appear, and soon a pore is formed showing the smooth margin of the corresponding mesh of the skeleton. As might be expected from this mode of perforation, which is not due to the presence of previously formed lines of weaker tissue like those along which the dehiscence of valves generally takes place, neither in the shape, size, number, nor position of the pores, nor their sequence is anything like regularity. In the material of the Kew Herbarium the number of pores is limited to few. But the fruits still bear the calyx-lobes and the decay of the epicarp is still in the first stage, whilst it is perfect in Zahlbruckner's specimen. Dr. Zahlbruckner denies the presence of any traces of insect-action or of wound-cork surrounding the pores. But both are present in the Kew specimens, and the only restriction I should like to make is as to their being caused by insects. I think it very probable, but I am not able to prove it from dry material. There are minute swellings on the surface, and a dissection shows that the epidermis had been injured and that wound-cork was formed subsequently from the nearest layers of the parenchyma. In other cases the wounds are larger, and their margins gape. They form a smooth rim which consists chiefly of a large-celled periderm. This periderm eventually permeates right through the epicarp, thus cutting out pores of a similar

appearance to those in the skeleton. It is evident that such perforations must accelerate the general decay of the epicarp, after which, however, all traces of them must disappear, as nothing is left but the netlike skeleton and the endocarp. Long before the decay of the epicarp has become general, the endocarp has split from the base towards the marginal ring of the capsule which bears the calyx-lobes. This splitting takes place in the same way as in *L. hypoleuca* and other species, but the tenderness of the outer tissues of their capsules causes them to tear as the valves expand, whereas the skeleton of *L. macrostachys* is sufficiently strong to resist that pressure. Thus the seeds are freed and find their way out, through the pores of the skeleton in *L. macrostachys*, through the ruptures in *L. hypoleuca*. The next question is if the normal dehiscence by two valves on the top takes place in *L. macrostachys*. So far as the material at hand and Hildebrand's observations go, it is not the case, though the anatomical structure is not of a kind to prevent it or to make it improbable. On the contrary, the very fruit which Dr. Zahlbruckner kindly sent, split after boiling, with a very slight pressure, along a line running from the persistent base of the style to the periphery of the top along which the normal dehiscence was to be expected.

The obliteration of the normal terminal dehiscence in combination with the peculiar development of the mesocarp and the mode of dissemination depending thereupon seems to deviate from what we find, for instance, in *L. nicotianaefolia* and *L. hypoleuca*. But I must now introduce another species which forms a connecting link in a striking way, *L. Gaudichaudii*, also a native of the Hawaian Islands, the close affinity of which to *L. macrostachys* cannot be doubted. Here we have a thin cartilaginous endocarp splitting loculicidally to the base, and a network of vascular bundles which though finer and less strong than in *L. macrostachys* acts in a similar way. It is strong enough to resist the pressure of the expanding valves, for a long time at least. The parenchyma decays more or less in the end, but without leaving such distinctly circumscribed pores, and its decay is more like that caused by ordinary maceration. The top, which is produced into a beak, opens either in the normal way, or it remains altogether entire or at least so long that meanwhile the dissemination by way of the decayed pericarp has already begun. With regard to *L. Gaudichaudii* we might say it is in a state of transition towards becoming indehiscent, a state which seems to have already been attained by

L. macrostachys. Thus the latter forms the extreme step in a direction which is indicated in the former. But, just as *L. macrostachys* is linked thus very closely to the allied species of *Lobelia* in spite of the obliteration of the dehiscence, so also the mode of dissemination which replaces that by way of terminal valves is the mere outcome of a structure which is perfectly congeneric to that found in the allied species. There is no element in it which would point to a generically distinct line of descent. It is rather a case of ultimate adaptation of a part of an organ to a particular biological function. There are many cases where species with dehiscent and indehiscent, with succulent and dry fruits, are placed in the same genus without anyone objecting to it, because the close natural affinity is evident notwithstanding the deviation in a single character which we know is exceedingly subject to adaptation to particular biological conditions. I admit that on the other hand genera are sometimes distinguished solely on such characters. But the expediency of this procedure is often very doubtful, and in other cases it might be defended because the two genera are characterized at the same time by the fact of their inhabiting quite distinct and often remote areas, or because the distinction is absolute, so far as our knowledge goes. Neither is here the case, and I am therefore of the same opinion as Mr. Hemsley, that the genus *Trematocarpus* can by no means be maintained by reason of its peculiar mode of dissemination.

Finally, I may mention that Hildebrand described the dehiscence or indehiscence respectively, and dissemination of *L. Gaudichaudii* and *L. macrostachys* in his Flora of the Hawaian Islands, pp. 236–7, briefly though not quite correctly in all the morphological details, in a way which amounts to the same as my interpretation of it.

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A MARINE FUNGUS.—In a recent paper[1] on 'Parasites of Algae' Mr. George Murray alludes to the doubtful nature of the records of higher Fungi actually inhabiting salt water. The following well-marked and probably widely-distributed instance may therefore prove of interest.

If the swollen fertile 'pods' of *Ascophyllum nodosum* be examined in early spring, they will be seen to be dotted over with numerous

[1] Natural Science, Vol. II, February 12, 1893.

minute black specks, just visible to the naked eye. On microscopical examination, these prove to be the perithecia of a minute Pyrenomycete of a *Sphaeria*-type. They are approximately spherical, and are completely immersed in the cortex of the Alga, penetrating only to a depth of four cells. The wall consists of a weft of very delicate hyphae, and a pore is formed at the surface. The asci are relatively large and contain eight elongated spindle-shaped spores, each being biseptate by an equatorial division. The mycelium is entirely confined to the swollen layers of the cell-walls of the host, and ramifies in great abundance in the highly mucilaginous wall-substance of the cells forming the inner tissue of the 'pod.'

We have then a definite example of the parasitism of an Ascomycetous Fungus on one of the Fucaceae. This particular form is probably of very general occurrence; in the neighbourhood of Plymouth I have never found *Ascophyllum* free from it, while material from Bangor showed it in equal abundance. It does not appear to occur on any of the species of *Fucus* growing in the same localities, nor again on *Pelvetia*, which extends much further up the tide-mark and may not be submerged for days at a time. It may, however, be suggested that *Ascophyllum* is, of all our seaweeds, modified as a *floating* Alga. Its very restricted range, between the limits of the neap-tides, together with its elongated buoyed stem, allow it to lie on the surface of the tide for the greater part of its existence, and hence it might be more exposed to the attack of floating fungus-spores than its more shrubby allies.

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Observations on Pitchered Insectivorous Plants (Part II[1]).

BY

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With Plates XIX, XX, and XXI.

II. Histology of Darlingtonia, Sarracenia, and Heliamphora, with remarks on adaptations for insect-catching.

THE degree of histological differentiation exhibited by the genera already treated of (*Nepenthes*, *Heliamphora*, *Sarracenia*, and *Darlingtonia*) is worthy of note. *Darlingtonia* and *Sarracenia* exhibit practically an equal degree of complexity in their hairs and glandular structures, though on slightly different lines of formation. *Heliamphora* again is very nearly related to *Sarracenia*, but in hair-distribution and gland-structure exhibits an interesting approach to *Nepenthes*, the most highly specialized of all. But while the first three genera possess an elaborate arrangement of hairs for bewildering and catching animal prey, the last is entirely

[1] For Part I, see Ann. of Bot. III, p. 253. Since this paper was written, the second part of Professor Goebel's Pflanzenbiologische Schilderungen has appeared. It has been considered advisable to leave the text unaltered, but footnotes have been added where such seemed necessary.

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devoid of them, at least inside the pitcher. The great complexity of the glands in *Nepenthes*, as contrasted with their comparative simplicity in the first three, more than compensates that genus for the absence of hairs. In describing the minute structure of the pitcher-surfaces I will use the expressive terms 'attractive,' 'conducting,' 'glandular,' and 'detentive,' applied to these by Sir Joseph Hooker in his Presidential address [1] to the British Association at Belfast. But, as I have already shown [2], external honey-glands are scattered over the leaves and even the stem in all the genera, forming an alluring or baited pathway for insects. I propose to speak of these as 'alluring glands,' and an area provided with them as an 'alluring surface.' Further, in view of the interesting researches of Hunt and Dickson on the glands of the corrugated rim of *Nepenthes* [3], and observations of my own as to their function, the term 'attractive' as applied to that genus will be extended so that we may speak of 'attractive lid-glands' and 'attractive marginal-glands.'

Darlingtonia. In this genus alluring glands occur over the entire outer pitcher-surface, and are not more abundant on the dorsal wing than elsewhere. When microscopically examined, they are circular or oval in outline (Plate XIX, Fig. 2); they appear to be, and have hitherto been described as being, one-celled [4], and their margin often shows a concentric ragged-looking line caused by the free edge of the cuticle, which is not continuous over the gland-surface. When vertical sections are made, each surface gland-cell is seen to be the uppermost of four (rarely five or six) sunk in the tissue of the leaf, the three upper being shallow and having thin transverse septa, while the lowest is large and goblet-shaped (Plate XIX, Fig. 1). The cell-contents have a dense finely-granular aspect, and are slightly yellow in

[1] Report Brit. Assoc. 1874.

[2] Nature, Dec. 25, 1884.

[3] Proc. Phil. Acad. Nat. Sc. 1874; Gardener's Chronicle, 1883.

[4] Zipperer, Beitrag zur Kenntniss der Sarraceniaceen, Munich, 1885. Goebel confirms my account.

colour, thus contrasting with the surrounding epidermal cells. In all, but specially in the goblet-cell, large refractive globules are present. On vertical section, it is evident that the cuticle, present as a protective layer over the ordinary epidermal cells, thins out round, and is absent from, the surface gland-cell, which, having only a thin cellulose wall, is free to discharge its secretion. At the upper arching part of the tube are the skylights, the nature of which has been fully explained by Zipperer[1]. There are no hairs over the exterior. The outer surface of the involute edge of the pitcher is richly studded with honey-glands, which are more complex than those just described, each being formed of 2–4 surface cells, and 4–6 times as many compose the entire gland (Plate XIX, Figs. 3, 4). They are also sunk in depressions of the epidermis, and are slightly covered by beautiful down-growing tooth-like processes of it, so that the whole greatly resembles the involute rim of *Nepenthes*. The inner surface of the involute edge is quite smooth to the depth of a quarter-inch, and destitute alike of hairs and glands, but below this is a narrow cincture of long, stiff, outstanding hairs, different from all other hairs of the pitcher.

As noted by Asa Gray, the bilobed flap secretes honey copiously on its inner surface from glands which are one-, rarely two-celled on surface view. But further at the free extremity hairs begin to appear, at first as minute protuberances of the epidermis, but towards the orifice these have so increased that there is a perfect forest of short, strong hairs directed towards the orifice.

The internal arched 'attractive' surface has long, stout, striated, downward-directed hairs, interspersed with two-, rarely one-celled glands (on surface view). There is an evident line of demarcation between the 'attractive' and 'conducting' surfaces, the latter consisting of cells each prolonged into a fine, sharp, striated hair. The junction of conducting and detentive surfaces is not clearly demarcated, the short hairs of the former being for some distance

[1] Op. cit. p. 25.

irregularly disposed among the long, delicate hairs of the latter.

I have not been able in this genus to watch insect-movements, but from our knowledge of *Sarracenia* the arrangements are evident. Insects alighting on any part of the tube, or crawling up from the ground, are tempted by the secretion of the alluring glands to run about till they reach the orifice. Whether entering the pitcher by the involute rim with its honey-laden surface, or by the honeyed flap, the cincture of stiff hairs inside the former, and the stout hairs of the latter, will act as a considerable obstacle to their easy return. Thus inclined to step inside the orifice, the abundant honey and the coloured skylights will tend to allay their fear, even though treading among the hairs of the attractive surface, which further incline them to move down. The extreme smoothness of the 'conducting' cells and their fine down-growing apices will combine, as in *Sarracenia*, to afford no foothold, and eventually the prey will get entangled in the long hairs of the detentive surface. Drude[1] states that *Darlingtonia* is provided with honey-glands, hairs, and digestive glands as in *Sarracenia*, but I have found no trace of the last, and Zipperer[2] says 'Drüsen sind auch hier keine vorhanden.' The insects caught in our greenhouses are almost entirely bluebottles, but earwigs and wasps are occasionally found. The late Miss Owen informed me that in a leaf, grown and examined by her, a slug was found near the bottom of the tube in state of advanced decomposition. The contrivances therefore which prove so fatal to insects may be equally destructive to slugs. It would be highly interesting to know if in the home of *Darlingtonia* the same happens.

Sarracenia. The different species of this genus exhibit some very interesting modifications both in general outline and histological arrangement. Hooker divides them under two heads: (1) those with the mouth open and lid erect, and which consequently receive the rain-water in greater or less

[1] Schenck's Handbuch der Botanik, Bd. I. p. 120.
[2] Op. cit., p. 26.

abundance; and (2) those with the mouth closed by the lid, into which rain can hardly, if at all, find ingress. But while in some respects this is a convenient physiological classification, I think it may be considered that the species now living group themselves round some extinct form most nearly related to *S. variolaris*. This view is favoured, alike from the standpoint of seedling pitcher-structure, as pointed out by Professor W. P. Wilson[1], from the structure of adult pitchers, and also from coloration and relative complexity of the floral parts. It will be seen, as we describe the minute anatomy, that *S. variolaris* is the most generalized form now known to us, and that from some simpler type one advancing line branches off to *S. flava*, *S. Drummondii*, and *S. rubra*; another leads to *S. purpurea*, and still another to *S. psittacina*. By viewing them thus we can, I think, bring into harmony their resemblances and differences in leaf-form, minute anatomy, and floral development, and they will accordingly be examined in the order just given.

But before treating of the species separately I may point out that the protective winter bud-leaves show many honey-glands, particularly over their outer surface. The question naturally suggests itself, Why should these be provided with an alluring surface? I think the answer is found if we view them as the earliest formed of the annual rosette of pitchered leaves which for protective purposes have become reduced in size and have failed to pitcher. They would represent, in fact, such a leaf as I have figured in Plate XVII, Fig. 8, of the Annals of Botany, Vol. III, but in a greatly more reduced state[2]. There it is compared with a pitchered leaf.

S. variolaris. The external pitcher-surface in this species is covered with short, stout, blunt hairs, each formed by the bulging out of an epidermal cell. These are directed outwards or obliquely upwards, are shortest and most scattered towards the base, and increase in size and regularity

[1] Proc. Philadelphia Acad. Nat. Science, 1888.

[2] Goebel adopts this view also, and his interesting observations on the relation of scale-leaves and foliage-leaves (Bot. Zeitung, 1880) may be said to establish it.

towards the orifice. This device undoubtedly will help to herd insects upwards, just as the internal hairs are arranged for impelling them in a downward direction, and a similar condition is slightly indicated in *S. Drummondii*. Among the hairs are many honey-glands of ordinary structure, viz. four subjacent and two smaller upper secreting cells. Along the ventral wing both hairs and glands are more densely massed than over the general surface, so that the front wing here is evidently developed as the special insect pathway. This is not the condition in all the species, as has erroneously been supposed. Towards the arched top of the pitcher, clear window-like areas occur quite resembling those of *Darlingtonia* in appearance and structure, as well as in absence of external and internal hairs and glands. Round these areas the hairs are so disposed that each is a fenced enclosure open only above, for while the lower and upper hairs are directed upwards, the lateral ones point obliquely towards the area. These areas therefore may act as temporary resting-places for smaller insects, allowing them time to overcome fear excited through the impediment offered by the hairs to their feet.

At the junction of the outer and inner epidermis, the honey-glands are very abundant; also the cells of the latter begin to project inwards as short, toothlike processes. These are continued round the interior to form the upper limit of the conducting area. The attractive area, which is here the inner surface of the arched lid, has strong hairs of moderate length (Plate XIX, Figs. 5 *a* and *a'*) and honey-glands. The junction of attractive and conducting surfaces is sharply marked to the naked eye, but microscopically examined it exhibits a very gradual merging of the one into the other. The transition from conducting to detentive surface is also gradual, and glands are found (Fig. 5) on both. These are rather sparingly scattered over the former, are specially numerous on the upper part of the latter, but entirely disappear towards the bottom of the tube.

It will now be seen that the adaptations for insect-catching

are of a rather elaborate kind in the above species. It alone is well provided with external hairs and with window-like areas, though both are shown in a less perfect manner in *S. Drummondii.* But the distribution of honey on the alluring surface is neither so widespread, so perfectly disposed, nor so abundant as in other species, so that my experience of it in the houses of the Royal Botanic Garden of Edinburgh as a rather inferior fly-catcher is probably a true index to its capabilities in its natural haunts. In making this statement I bear in mind that it was this species which Drs. Macbride and Mellichamp have described so carefully, but compared with *S. flava* and *S. Drummondii* I have always found it to be inferior.

My knowledge is still imperfect regarding gland-secretion. That the alluring and lid-glands, as also those on the upper part of the conducting surface, secrete a sweet juice, any one can satisfy himself. But the function of the glands covering the lower conducting and detentive surfaces is more difficult to explain, as the secretion is not sweet to the taste. In view of the wide distribution of the honey-glands over each leaf and parts of the flower, it appears to me, from an evolutionary standpoint, quite a likely hypothesis to suppose that all the internal glands may originally have secreted a sweet attractive juice. In structure the internal glands resemble each other, and also the external ones. Now in *S. variolaris* no separation or isolation of the internal glands into areas is observable, though, as already stated, they are rather few in number on the conducting, but abundant on the upper detentive region. It is quite probable therefore that some simpler form is now lost to us which exhibited a uniform distribution of internal glands, all secreting honey. The advantage of such an arrangement, especially if correlated with a less specialized hair-development, is manifest. But *S. variolaris* and the other species that are provided with glands in the lower conducting area or parts beneath, secrete a juice which, according to Mellichamp[1], is astringent, and

[1] Gard. Chron., June 1874.

hastens decomposition, according to Drude[1] is probably acid and causes a true digestive change. In *S. crispata*, a probable hybrid between *S. flava* and *S. rubra*, a very large quantity of sweet juice is secreted by glands on the top of the conducting surface, beside that secreted by the lid-glands. This form has proved, in the houses of the Edinburgh Botanic Garden, the most efficient fly-catcher; but not only so, some constituent of the secretion is of a decidedly acid nature, and at once causes litmus-paper to become red. These glands are continued down the ventral part of the conducting-area to fully half its depth, and if we take into consideration the occurrence of them so far down, as also their excretion of a sweet and acid juice, the hypothesis which I have advanced above does not seem unwarranted. The lower glands of the tube secrete even in young unopened pitchers, a juice which Mellichamp well describes as being mucilaginous and astringent. If, then, the lower glands in simpler types of the genus once secreted a sweet juice, its place has been entirely usurped by the more useful insect-wetting, and perhaps digestive, juice.

S. flava. This, the strongest growing species of the genus, is richly provided with alluring glands disposed in an interesting manner. Since the strong vascular bundles which traverse longitudinally the external pitcher-surface form projecting ribs, lines of glands occur along these as well as on the dorsal wing; many baited pathways therefore, supplemented by glands scattered less abundantly over the surface, allure insects upwards. Fresh healthy pitchers examined about the beginning of June show transparent droplets of honey exuding from them, and the secretion may be continued into autumn, or cease to a large degree during summer and be renewed in September. Alluring-glands closely stud the external margin of the pitcher-orifice. But insects often pass up the ventral rib on the outer lid-surface, and accordingly its margin has a perfect cincture of glands from which a sweet secretion pours forth copiously. I am surprised that this honey-cincture does not seem to have

[1] Op. cit. p. 136.

been noticed before, as it forms in the sunshine a most conspicuous glistening marginal belt, and also allures insects most powerfully to the inner lid-surface, as has often been verified by observation. The other species show the same massing of glands, but none so perfectly. The attractive or inner lid-surface, has hairs similar to those of *S. variolaris*, but larger. Many glands are scattered among these, but further, as mentioned in the probable hybrid between this and *S. rubra*, a long ventral continuation of glands, secreting a sweet but acid juice, runs down into the conducting surface.

The hairs on the conducting and detentive surfaces resemble corresponding ones from *S. variolaris*, but on a larger scale.

While a moderate number of glands occur on the conducting, few or none are met with on the detentive surface.

S. Drummondii. Alluring glands are more sparingly distributed over the leaf-exterior of this than of any other species. The outer lid-surface has all or most of the epidermal cells raised into boss-like swellings directed slightly upwards, so that in this as in *S. variolaris* an attempt at formation of external upward-directed hairs is shown.

The attractive (inner lid) surface bristles with very long stout hairs, greatly indurated at the base, and carries attractive glands of ordinary structure. The conducting surface has the cells produced into downward-directed processes—which are pretty long above, but become greatly elongated below, as the detentive surface is reached. Attractive glands are found plentifully over its upper area, but these become finer till they quite disappear from the lower two-thirds of the area; their place is taken however by stomata set in the centre of a group of cells. On the detentive surface short and relatively delicate hairs are developed from many of the cells. Some stomata are also present. There is no proof as yet that the stomata are for water-excretion, but their presence in this species qualifies Zipperer's assertion[1] that stomata are absent over the inner surface of the tubes.

S. rubra. Alluring glands are pretty abundant over the

[1] Op. cit. p. 31.

external surface, 3 to 3·5 being the average over a square millimetre, but they are decidedly more numerous on the dorsal flap, which may show 5 to 6 over a similar area. Hairs pointing very irregularly, the majority more or less upwards, are also found over the tube, and become specially abundant and strong on the lid; but these, so far as I can see, will perform no special part, though they may represent a rudimentary state of the more perfectly developed condition seen in *S. variolaris*. The attractive surface is practically identical with that of *S. flava*, the hairs being only slightly smaller. Both species agree also in having a triangular-shaped ventral prolongation of the attractive surface running down into the conducting region. On the conducting surface are very fine closely-set hairs, but there is an entire absence of glands. The latter are also absent from the detentive surface, the hairs of which are extremely fine for their length. It is to be noted, then, in this species, that glands are only present on the attractive surface.

S. purpurea. Very careful descriptions of this species have been given by Voigt, Hooker, and others, which stands alone in having a special glandular surface in the tube. In addition to many alluring glands, there are on the outside numerous strong, stiff, blunt hairs, rather irregularly disposed, though most point upwards. I need not here repeat the descriptions of previous workers, except to emphasize the fact that all the glands of the plant resemble each other structurally. In Pl. XIX, Fig. 6, illustrations are given of the areas and of the hairs occurring on these to aid in descriptive comparisons of a hybrid of this species with its parents.

S. psittacina. This is the most aberrant of all in its histology as in its general morphology. The alluring glands agree in number and disposition with the species just discussed, but they are extremely abundant along the outer side of the inflexed rim, so as to resemble *Darlingtonia*. The attractive surface differs from that of the other species in the great length and delicacy of its hairs, which resemble most the detentive hairs of *S. rubra*. The honey-glands found with these are

very abundant. The junction of attractive and conducting surfaces is very abrupt, and forms a line running round the interior of the hood, on a level with the pitcher-orifice. The conducting surface, which is a narrow zone from $\frac{5}{8}$ to $\frac{3}{4}$ inch in depth, has few or no glands. Along its upper region each cell is produced into an extremely short downward-growing point; lower down, however, the cells gradually lengthen until they resemble similar cells of *S. purpurea*. The detentive surface includes the greater part of the pitcher-tube. Its hairs are very long, and, in striking contrast to the conducting surface, it is abundantly supplied with glands over its upper third. In no other species are there so few on the conducting, and so many on the upper region of the detentive surface.

In all the species, then, an amount of sweet juice is secreted corresponding in quantity to the number of alluring and attractive glands. Sir J. Hooker suggests that it is probably ground-game which is led up to the pitchers, and while this may be true to some extent, in our greenhouses flying insects almost entirely are caught, and these consist in about eighteen cases out of twenty of bluebottle flies, with an occasional earwig, wasp, or house-fly. These alight on some part of the tube and gradually crawl up to the pitcher-mouth, sipping the honeyed juice as they go. It may be that in their native haunts running insects or even, as in the case already mentioned of *Darlingtonia*, slugs form part of the prey, but that flying insects are as easily captured is undoubted[1].

If we now make a short comparative review of the six species it will be manifest that *S. variolaris* is a central type from which the others radiate off. I do not necessarily mean by this that they have been derived from it by evolutionary modification, but rather, that in process of evolution the

[1] Since writing the above I have had the opportunity of examining *S. purpurea* in the New Jersey Swamps, and find that ground-game, notably ants, are largely caught by the pitchers. Flying insects and slugs are not uncommon, and though bulk for bulk they may yield a considerable food-supply for the plants, Hooker's supposition appears correct for this species. In one specimen examined a large nest of ants had been established in three of the older and rather dry brown leaves, just beneath the reddish green leaves that were actively catching prey.

above-named form is one that has deviated least from a common ancestral type. Thus its leaves incline to grow obliquely upwards, while on the one hand those of *S. purpurea* and *S. psittacina* are nearly or quite horizontal, and on the other those of *S. flava*, *S. Drummondii*, and *S. rubra* all grow erect, or nearly so.

Next, in *S. variolaris* the processes from the cells of the conducting surface are pretty short, and both conducting and detentive surfaces are glandular, particularly the upper part of the latter. In *S. psittacina*, the processes are still shorter, and resemble closely those of *S. purpurea*, while a great massing of glands occurs at the top of the detentive surface. If we imagine the upper part of the detentive surface, in either of the two last, to have lost the hairs and to have retained only the closely-set glands, we should get the special 'glandular' surface of *S. purpurea*, and that this is a modification of the detentive surface appears probable when we examine hybrids of the species. Again, starting from *S. variolaris*, we pass to *S. flava*, with a few glands on the conducting, and still fewer on the detentive surface; then to *S. Drummondii*, with a few on the upper part only of the conducting surface, and none on the detentive surface; lastly to *S. rubra*, where both conducting and detentive surfaces are devoid of glands. The hairs in the three last likewise point to a similar deviation. That these highly-specialized leaves have been derived from some simpler type cannot be doubted, that they are all inhabitants of N. America points strongly to a common geographical origin, that they all show so many points of similarity in histology and morphology, with corresponding physiological adaptations, indicates a common line of development, and that they tend to vary in minor morphological and physiological details leads us to believe that connecting types have been lost.

Heliamphora[1]. From the leaf-base upwards this genus shows externally many honey-glands, an average of 3 to the square mm. being encountered over the general surface, but

[1] I am indebted to Harry Veitch, Esq., for excellent fresh specimens of this rare plant.

on the dorsal flaps there may be as many as 4–6. These lower glands resemble corresponding ones of *Sarracenia*, but towards the pitcher-orifice they become more complex, each being made up of 8–12 cells. Interspersed amongst them are upward-directed bifurcated hairs, figured by Zipperer. The outer surface of the rim is well provided with glands and hairs.

In young pitchers the inner lid-surface has numerous rather small glands, but in adults many of these become of great size and complexity, consisting of from 30–70 cells. Each gland lies in a depression bounded by thick-walled neighbour-cells, the upper of which may overhang it, so that they are quite like many *Nepenthes* lid-glands (Pl. XIX, Fig. 8). The hairs also which grow out among them are very delicate, or may be entirely absent at the top of the lid (Fig. 7). This area is sharply marked off from a region that must be regarded as a union of the attracting and conducting surfaces of *Sarracenia*, since it is provided with glands, and with hairs of very variable size. Succeeding to this is a smooth area (Fig. 7*c*.), which in the absence of glands and hairs, and in the wavy outline of the epidermal cells composing it, completely reminds one of the conducting surface in *Nepenthes*. The lowest area bears short, greatly-thickened epidermal hairs, which Bentham has figured and described. This is the true detentive surface. That *Heliamphora* should be a good fly-catcher might be anticipated from the quantity of honey secreted by the alluring glands, and even more by the attractive glands, where the dried secretion often appears as a white sugar-like concretion on the surface ridge; from the number and length of the conducting hairs; and from the presence of a smooth conducting surface. Bentham in his memoir inclines to regard the number caught as insignificant, but from specimens examined in the Kew Herbarium it appears in this respect to compare favourably with any *Sarracenia*. From the foregoing one learns how curiously the peculiarities of *Sarracenia* and *Nepenthes* are blended in this interesting type. I can scarcely suppose that this is accidental—rather may we see in it an example of true genealogical relationship. The extremely isolated distribution

of the genus suggests the former existence of some connecting types, alike on the *Nepenthes* and *Sarracenia* sides.

III. General Morphology and Histology of the flowers of Darlingtonia, Sarracenia, and Heliamphora.

All botanists have traced in these three genera so many and close similarities as well of pitcher as of flower-structure, that they have agreed in grouping them into one order; but while *Darlingtonia* and *Sarracenia* have very close floral relations, *Heliamphora* deviates widely. The complete pentamerous and gaudy flowers of the first two contrast with the last, which bears 4 rarely 5 white or rosy sepals and a tricarpellary pistil maturing winged seeds, but in these very points decided affinities are established with Nepenthaceae. If the ordinal descriptions given in De Candolle's Prodromus by A. de Candolle for Sarraceniaceae and by Sir J. Hooker for Nepenthaceae be compared, the remaining characters will be found to agree fundamentally. To return to this at a later stage, we may now describe and compare the floral parts of each genus.

(*a*) **Darlingtonia.**

While working at the lid-glands of *Nepenthes*, on speaking of its floral structure to Mr. Lindsay he drew my attention to the fact that from the glands, which Hooker mentions as occurring on the sepals, a very copious flow of honey takes place during flowering. The hint thus obtained being followed up, has yielded me some interesting results. Hooker[1] says of *Darlingtonia* that he was struck with 'a remarkable analogy between the arrangement and colouring of the parts of the leaf and of the flower. The petals are as highly coloured as the flap of the pitcher, and between each pair of petals is a hole (formed by a notch in the opposed margins of each) leading to the stamens and stigma. Turning to the pitcher, the relation of its flap to its entrance is somewhat similar. Now we know that coloured petals are specially attractive organs,

[1] Brit. Assoc. Address, Belfast, 1874.

and that the object of their colour is to bring insects to feed on the pollen or nectar, and in this case by means of the hole to fertilize the flower; and that the object of the flap and its sugar is also to attract insects, but with a very different result cannot be doubted. It is hence conceivable that this marvellous plant lures insects to its flowers for one object, and feeds them while it uses them to fertilize itself, and that, this accomplished, some of its benefactors are thereafter lured to its pitchers for the sake of feeding itself!' This is true even in a more intimate way than Hooker imagined, not only of *Darlingtonia*, but of every genus of pitchered insectivorous plants.

The sepals of *Darlingtonia* are provided with honey-glands like those of the outer pitcher-surface. The presence of these undoubtedly proves 'that this marvellous plant lures insects to its flowers for one object, and feeds them while it uses them to fertilize itself, and that, this accomplished, some of its benefactors are thereafter,' by the rich provision of food already described, 'lured to its pitchers for the sake of feeding it.'

(*b*) **Sarracenia.**

The three bracteoles and five sepals of all the species develop many honey-glands like those of the pitcher, and though I have only observed a sweet secretion on four or five out of about two dozen flowers examined, this may be explained by differences in greenhouse growth as compared with free growth in their native haunts; or the secretion may exude only for a limited time. This point deserves further investigation. The petals vary greatly in colour and size, being small and of a dull unattractive green or greenish-yellow hue in *S. variolaris*, largest and of a pale greenish-yellow in *S. flava*, small and greenish-crimson in *S. rubra*, large and of a deep crimson-purple in *S. Drummondii*, large and purplish-red or brick-red in the petals, and more or less also in the sepals in *S. purpurea* and *S. psittacina*. Thus the development and distribution of flower-colour in calyx and corolla indicates a relation in the species similar to that which we

have attempted to trace in the pitchers. In all, the epidermal cells of the ovarian surface have undergone repeated divisions, and have swollen out into minute glassy beads or tubercles (Pl. XIX, Fig. 11), from which a quantity of rich nectar exudes, before, during, and for some time after blossoming. This, as we will show, is evidently of great use in the pollination of the flower.

(*c*) **Heliamphora.**

On the flower-stalk are two or three sheathing sessile bract-leaves. These display a considerable abundance of glands like those of the outer leaf-surface. The same remark applies to the four or five rosy-white sepals (Pl. XIX, Fig. 10). I have had no opportunity of examining the structure of the ovarian surface, though Bentham describes it as being hairy.

To sum up the genera: all have 'attractive' glands on their sepals, and *Sarracenia* and *Heliamphora* also on the bracts, these secreting what might be called extra-floral nectar. *Sarracenia* further has a huge glandular ovarian surface for the secretion of intra-floral nectar.

IV. Arrangements for Pollination in the Flowers of Sarracenia.

As previously mentioned (p. 413), flying insects of the dipterous or hymenopterous groups are those mostly found to frequent, and to be caught in, the pitchers. But the species caught are not the most specialized flower-pollinators; the bluebottle and wasp having smooth bodies and feebly-developed leg-hairs. A special arrangement exists, therefore, to suit their condition[1].

When a flower has nearly opened the stamens begin to dehisce, and as the blossom has a pendulous position the pollen from the stamens is showered down into the umbrelloid style-cavity below. But about this time the warted bead-like ovarian surface exudes large drops of sweet juice, which increases in quantity as the stamens continue to dehisce,

[1] When I prepared the account given above I was not aware Dr. Masters had published a description in the Gardener's Chronicle, vol. xv (n. s.), 1881, p. 628.

till it oozes down among the filaments and anthers, washing with it the pollen-grains. It then accumulates in the umbrelloid cavity, and forms there a nectar-bath of pollen. Now each petal has a narrowed base inserted into the receptacle, close against and beneath the stamens; the petal then widens out and passes down straight, or rather obliquely, for about half an inch to an inch (varying in different species). It then bends outwards, and, expanding considerably, hangs over as a pendant banner between the two adjacent incurved angles of the umbrella-shaped style. Such being the course and shape of each of the five petals, it follows that an insect that has crawled up the flower-stalk or alighted directly on the outer calycine whorl will reach the interior most readily by passing through the opening between two adjacent petals, but to do this it must step first on to the outer surface of the umbrelloid style, and, guided by the hairs of the style-surface, which point towards the stigmatic knobs, it must creep round the edge of the style at or near the knobs, so that it must in many cases touch them. If, therefore, an insect has already visited a flower, the nectar-soaked pollen that is smeared over its body can scarcely fail to be rubbed against the stigma. Reaching the interior of the style, it will again revel in a nectar-bath and again be abundantly smeared, but on leaving it can most easily get out by rounding the edge of the style considerably below the stigmatic knob, since the latter is in the way of its freely mounting out, and the style-arms themselves are often incurved.

I have not been able to satisfy myself as to when the stigmas mature. The stamens, as above noted, begin to dehisce before or just as the flower opens, but at that time the stigmatic surfaces do not appear more or less fit for pollination than at a later period. Some gardeners have experienced difficulty in getting the Sarracenias to seed under cultivation even when cross-fertilization was attended to, but the fact that many hybrids have been obtained, indicates the possibility of definite results if fertilization could properly be effected. It occurred to me that the possible explanation was

that gardeners, in attempting to fertilize, may have detached dry pollen which had not been nectar-smeared, and this when applied to the stigma may have been so dry as to render it inoperative. Speaking on this subject to Mr. Pope, of Glasnevin Garden, Dublin, he at once stated that he always selected nectar-smeared pollen to effect fertilization. An interesting line of experiment is here suggested, which may bear practical results.

V. Histology of Nepenthes, with Remarks on Adaptations for Insect-catching.

This section of the present paper may best be understood if a general review of the comparative morphology of the species be first given.

N. ampullaria and *N. Lowii* are types which point to a simpler condition of pitcher from which they have both been derived, and which in form would approach the Sarracenioid alliance. Resembling *N. Lowii* in being a tube with simple non-corrugated or slightly corrugated rim, such a form would foreshadow *N. ampullaria* in the rudimentary condition of its lid, the absence or small number of attractive lid-glands, and the simple unconstricted shape of the pitcher. But with such a primitive form we must associate a higher degree of histological differentiation than is shown even by *Heliamphora*, the most specialized in its pitcher of the Sarracenioids, for the presence of regularly disposed attractive marginal glands in the leaves that appear after the cotyledons is of considerable importance.

As indicated in seedlings, such a primitive type would have an uninterrupted lamina, would be elongate-tubular in shape, with a simple pitcher-margin ; the lid would be of small size, and would stand upright, or even be inclined back at an angle in mature pitchers. The lamina would have flat glands completely exposed, the simple margin would be encircled with attractive marginal glands sunk in fossae, and the whole interior of the pitcher-cavity would show glands similar in structure to the laminar ones, but differing in their secretion.

That this is no mere hypothesis is proved alike in the development of seedling-leaves and in the young state of adult leaves, for in all that I have examined the marginal glands first appear and attain the largest size; simultaneously with them, though greatly simpler in structure, the internal 'digestive' glands; thereafter appear a few alluring glands on the general leaf-surface; and last, when present, the attractive lid-glands are developed.

But a study of the species of *Nepenthes* at present known leads one to conclude that, excepting two or three which have early branched off from the primitive type, all can be naturally connected with each other by easy gradations. I shall now shortly pass these in review, and note points of interest in each.

In *N. ampullaria* the pitcher is tubular, its broad corrugated collar is so abruptly curved into the pitcher-cavity as to be quite parallel to its walls; the lid is tilted back at an angle of about 110°–130°. Only in one or two instances have I observed alluring glands on the outer lid-surface; the marginal glands are of small size and ampullate in shape; the conducting surface, represented by a narrow glabrous band internally at the top of the tube, is functionless, and small digestive glands thickly cover the whole inner cavity[1].

In *N. Hookeri* the pitcher is tubular or globose; its orifice is surrounded by a narrower collar than in the last, and the collar is inclined at an acute angle to the cavity; the lid is greatly larger, and is vertical or slightly inclined over the pitcher-mouth. A few alluring glands are present, and the inner lid-surface may either be devoid of glands or have from ten to thirty along the grooves of the lid. The marginal glands are ampullate, and decidedly larger than those of the last species, though the conducting and digestive surfaces remain the same.

In *N. Rafflesiana* the pitcher is tubular and globose or cornucopia-shaped; the corrugated collar is relatively narrower

[1] It will be seen that even in this species a marked advance on the primitive type is shown in the possession of so deep a corrugated rim, which imperfectly functions, instead of the more specialized conducting surface of the higher forms.

than that of *N. Hookeri*, but its teeth are more strongly developed, and the collar rises up as a posterior neck-like elongation of the pitcher; the lid is large, and rarely vertical, more often inclined inwards over the cavity. The inner lid-surface has attractive glands in abundance along its lateral grooves, but these are scarce or absent at the sides and along the middle. The marginal glands are ampullate, but larger and with a nearly blunt nipple as compared with the smaller glands of *N. Hookeri* and *N. ampullaria*, which have well-marked nipples. The conducting area is here a rim about a quarter of an inch wide, which expands behind into a deltoid space about three quarters of an inch to an inch in depth in pitchers of average size. The digestive surface shows larger glands than in either of the preceding.

From *N. Rafflesiana*, or some nearly related form that may now be extinct, several lines of modification proceed. Thus *N. bicalcarata* has pitchers greatly like the ground ones of *N. Rafflesiana*. The elongation of the collar posteriorly, and the shape of its teeth, the size and shape of its marginal glands, and the disposition of the conducting and secreting surfaces, all conform to the last type. It shows an advance in the abundant development of attractive lid-glands and the great elongation of the posterior teeth of the collar into two long curved processes, the use of which has been very ingeniously accounted for by Mr. Burbidge.

From nearly the same starting-point another line can be pursued which leads us to *N. Veitchii*, with tubular ovoid pitchers having a hairy external surface, broad frill-like collar, and incurved lid. Its entire glandular arrangement corresponds closely with that of *N. Rafflesiana*, though showing advance in several details. *N. Celebica*, with its very large lid-glands, broad collar, and rather deeper conducting surface, advances again beyond *N. Veitchii*. I can scarcely help regarding *N. Curtisii* as a hybrid between *N. Veitchii* and some dark crimson form with narrow elongated pitcher, at present unknown to us. *N. villosa*, *N. Harryana*, and *N. Edwardsiana* represent types of advancing specialization along the line of *N.*

Veitchii, in which the pitcher-rim has not only enlarged greatly, but gradual increase in the size of the lid and of the marginal glands, increase in depth of the conducting surface and in strength of the pitcher-substance, have all proceeded simultaneously, and culminated in the last-named, which may fairly be viewed as the most highly-developed species of the genus. *N. Rajah* and *N. Boschiana*, in the relatively small size of the marginal glands and feeble development of conducting surface, show close relationship to *N. Veitchii*, though in the size of the pitcher and of its lid and rim as well as in coloration, they mark a decided advance on it.

Another and a very interesting line passes to the isolated species *N. echinostoma*, which, in the possession of a functional conducting surface as well as in the disposition and size of its glands, is more specialized than *N. Rafflesiana*, but is singular in having a reduced reflected lid with large glands, and in having the ridges of the collar split up into narrow tapered processes that curve round the orifice of the pitcher. Each carries a marginal gland at its free extremity. Another line leads us to *N. sanguinea* and *N. Northiana*, that show strong vegetative growth and richly coloured pitchers. These pitchers show richly honeyed lids, waved collar with cylindrical marginal glands, and conducting surface of considerable depth.

Again, *N. Bongso*, by its reduced vegetative state, narrower rim, less elevated posterior beak, and other peculiarities, leads us to a series such as *N. tentaculata*, *N. gracilis*, *N. alata*, and *N. distillatoria*, all characterized by an abundance of lid-glands, narrow reduced collar, that carries correspondingly reduced marginal glands, and gradually deepening conducting surface, which in the last is from one half to two thirds the depth of the pitcher. *N. Pervillei*, *N. laevis*, and *N. Vieillardii* appear to be in structure divergent forms from these. The closely related species, *N. Phyllamphora*, *N. khasyana*, *N. Madagascariensis*, and *N. Kennedyana*, are all highly evolved forms in which the long tubular pitchers, richly honeyed lids, large sausage-shaped marginal glands, and deep conducting surface, suggest

a common origin, though their geographical distribution is now so diverse.

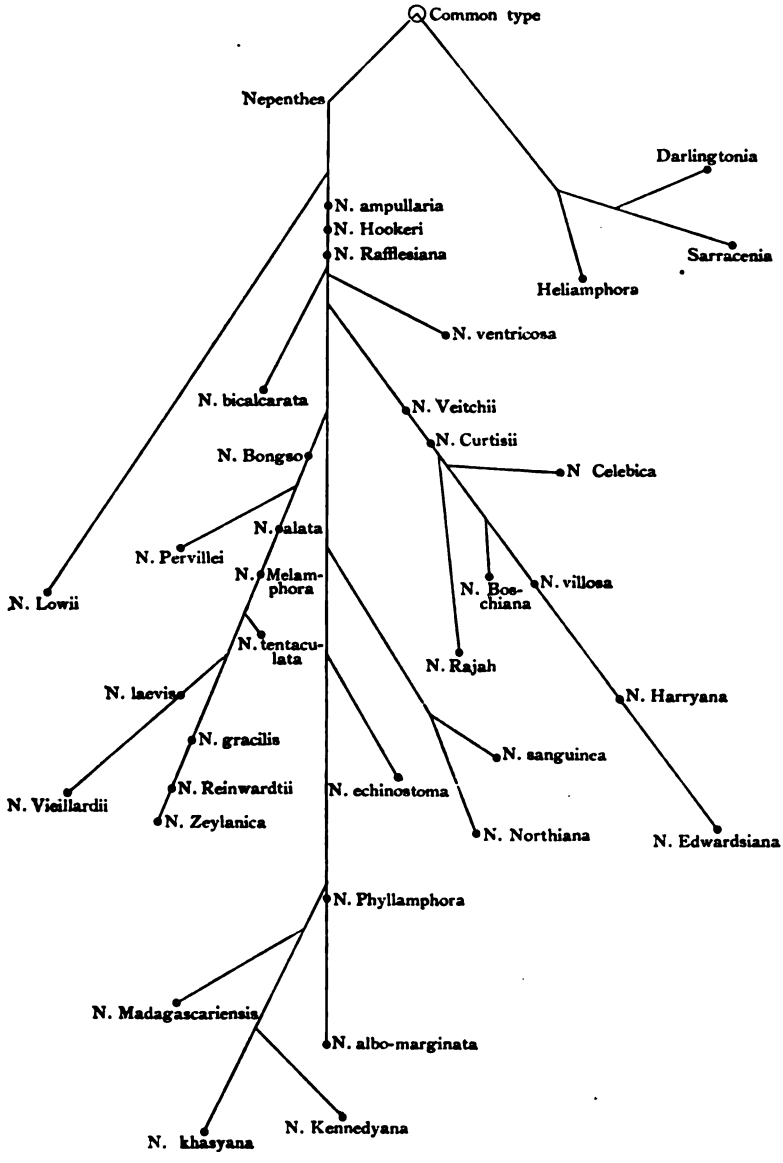
Lastly, *N. Lowii* is very difficult to connect with other species, and seems early to have branched off from a primitive type.

On page 425 I have arranged a chart which gives the structural relations of the species, and though it would be rash to assert that it is genealogically correct in every connexion, I think it may fairly and approximately be taken to represent the leading lines of development followed in the evolution of the species. As explained later on, a complicating factor is introduced in the ease with which *Nepenthes* hybridize, and there is some difficulty in ascertaining what may be regarded as species capable of perpetuating themselves.

The more general consideration of the histology of *Nepenthes* may now be entered upon.

(*a*) *Vascular supply.* A special feature of the genus *Nepenthes* is the intimate relation which the vascular system bears to the glands. In *Sarracenia* and *Darlingtonia* the glands are never connected with the vascular system; in the more complex lid-glands of *Heliamphora* I have repeatedly observed that vascular bundles pass behind or appear to end in them, but my material has been insufficient to assure me.

In *Nepenthes* every gland is directly seated on a vascular bundle, or a vascular diverticulum ends beneath it. This fact is beautifully demonstrated if pitchers are macerated in the manner recommended in Part I of this paper (Annals of Botany, Vol. iii, p. 254), and are gradually dissected from without. A xylem and phloëm portion are readily distinguishable by their respective elements in any bundle near the base of an alluring, attractive, or digestive gland, but the direct junction consists of slightly elongated cells with dense protoplasmic contents. The vascular supply to the marginal glands is, as we might expect, most complex. Each ridge of the corrugated collar is traversed internally by a strong bundle given off from a common encircling one, and this,


Common type
Nepenthes
Darlingtonia
N. ampullaria
N. Hookeri
N. Rafflesiana
Sarracenia
Heliamphora
N. ventricosa
N. bicalcarata
N. Veitchii
N. Curtisii
N. Bongso
N Celebica
N. gracilis
N. Pervillei
N. Melamphora
N. Boschiana
N. villosa
N. Lowii
N. tentaculata
N. Rajah
N. laevis
N. Harryana
N. sanguinea
N. Vieillardii
N. Reinwardtii
N. echinostoma
N. Zeylanica
N. Northiana
N. Edwardsiana
N. Phyllamphora
N. Madagascariensis
N. albo-marginata
N. Kennedyana
N. khasyana

when it approaches the gland, splits up into five or six branches that run down close against its side and near to its free end. Each gland, therefore, is surrounded by a circle of bundles (Plate XX, Fig. 13).

(*b*) *General mesophyll-tissue.* This has been fully described by several authors, and consists of large loose cells interspersed with many long spiral cells, such as are so abundantly found in the stem. The latter must give a high degree of tenacity and flexibility to the plant. But, as in the Sarracenias, the mesophyll-cells beneath the inner pitcher epidermis show secondary deposits of thickening matter on the primary membrane in which unthickened spot-like areas are left. The centre of each is traversed by one, rarely two, pore-apertures, that can be easily demonstrated under a Zeiss D objective by proper light manipulation. For rapid transference of food-material the appropriateness of these being on the inner side, next the pitcher-cavity, is evident.

(*c*) *Epidermal modifications other than glands.* At least three varieties of hair may be encountered over the outer surface of many species. Most common is the short, flat, brown hair made up of a shallow stalk-cell with five depressed cells at the top. Frequent with these (as in *N. Veitchii*, *N. villosa*, &c.) are compound tufted hairs, each made up of a stalk varying in length, which gives off five to fifteen arms. It is to the presence of this type of hair that the pilose or hairy aspect of many species is due. On the pitcher and outer sepal-surface of various species (e. g. *N. villosa*), are long, simple, upward-directed hairs; while those of *N. bicalcarata* are short, multicellular branching processes, the cells of which are jointed to each other in an oblique manner.

Ordinary stomata occur on the under laminar surface, and are often very numerous. Peculiar 'minute reniform transverse excrescences' (Hooker), first noticed, so far as I am aware, by Oudemans[1], are referred to as follows by Wunschmann[2].

[1] De Beker-planten, Amsterdam, 1864.

[2] Ueber die Gattung *Nepenthes*, Berlin, 1872, p. 21.

'In this place I have to mention peculiar formations the existence of which I intimated before (p. 19), and which are only found on the wax-covered portion of the inside of pitchers. These are one-celled formations of peculiar form rising obliquely over the flat epidermis. The outer partition, which rises in a very small angle over the even part of the epidermis, is vaulted in the form of a saddle; there, where it begins to descend, the cells are spread in pretty large number in the epidermis, and lie always so that the saddle-like depression is directed towards the bottom of the pitcher. They are only distinctly to be recognized after removal of the waxy covering by which they too are covered, and which after melting in boiling water, or application of alcohol, fastens itself generally in the depressions. These cells have no glandular character at all, and the supposition which Oudemans occasionally expresses, viz. that possibly through them the secretion of the waxy granules is effected, appears to be unfounded, because the granules are spread irregularly all over the outer skin and are by no means to be found in greatest numbers in the immediate neighbourhood of the cells. These are evidently formations to be put with the one-celled hairs, and are only remarkable in so far as they are broader than high, so that their broad diameter is twice as great as their length.' These stud the conducting surface of all species in which this is of any depth. Dickson suggested that they might be modified stomata, since each 'transverse excrescence' was shaped like a stomatic guard-cell. He did not however find direct proof. By careful examination of several forms, most successfully of *N. hybrida* (*N. khasyana* crossed by *N. gracilis*) and *N. albo-marginata*, I have found in adult pitchers every transition between these and perfectly formed stomata. Reference to developing pitchers furnished verification of the observations on adults. While the pitcher of *N. hybrida* is still only one and a half or two inches long, the formation of stomata can be seen to proceed quite normally. Later they begin to be obliquely placed, and in pitchers about two and a half or three inches long,

each stoma has one guard-cell hid from view, along with the stomatic orifice, by the excrescent and parallel position of the outer cell. Each excrescence is therefore a stoma so placed that one of its guard-cells protrudes next the observer, while the other is pushed inwards. It at once occurred to me that the conducting surface seen in many *Nepenthes* might represent a water-stomatic region, and by inspection of undisturbed pitchers at different hours of the day I found that a considerable supply of liquid exuded from large pitchers with deep conducting areas, such as *N. khasyana*, &c. The amount was greatest in the morning, to judge from the size and number of exuded drops. Now Griffith[1] and Hooker[2] have likened the pitcher of *Nepenthes* to an enormously developed terminal water-gland of a leaf, and the conditions now indicated verify this. I hope in time to conduct experiments on the excretion of water, as the possibility presents itself of these modified stomata being the source of most of the liquid found in the pitchers.

(*d*) *Glandular modifications of the Epidermis.* These I will speak of according to their position and use, as (1) alluring stem-glands, and (2) alluring leaf-glands, both secreting a sweet juice intended to decoy insects to the pitcher-orifice; (3) attractive lid-glands, and (4) attractive marginal-glands, both exuding a sweet juice, the latter particularly relished by insects, and so placed as to induce them to step on to the inner pitcher-surface; (5) digestive glands, which, as already pointed out, may either be spread over the whole interior, or be restricted to the lower part of it.

Alluring stem- and leaf-glands. These in their simplest condition are composed of a flat layer of columnar epidermal cells, with clear protoplasmic contents, and two subjacent layers of angular epidermal cells, lying upon two layers of clear bead-like cells, beneath which the terminal cells of the vascular bundle end (Plate XX, Fig. 13). In the growth of three sets of seedling plants that I have examined, these

[1] Posth. Papers, Vol. ii, p. 77.
[2] Trans. Linn. Soc. Vol. xxii, pp. 415–424.

alluring glands always appear earlier than the lid-glands, as a few may be encountered on the fifth–eighth leaf, while the lid-glands are absent until the ninth–twelfth foliage-leaf has appeared. They are distributed in each species to a varying extent over the stem, petiole, mid-rib, under laminar surface, tendril, outer pitcher and lid surfaces. After examination of all the species, extremely few have been found on the upper laminar surface, except in three species soon to be touched on, and there are evident reasons for this. It may be laid down as a general rule that they increase in number as one passes from stem to outer pitcher-surface, and on the latter they are most abundant along the dorsal wings and near the pitcher-orifice. In *N. Veitchii*, while abundant on the under laminar surface, they are densely set at the base of the petiole as if to tempt insects to pass from the stem to the leaf. This is also true to a great extent of *N. Northiana*. The deviations which they show from the simple flat type already described are highly interesting. When placed on the outer surface of the lid or pitcher they much resemble attractive lid-glands, with the addition that a covering flap of epidermal tissue varying in extent grows over them, or more commonly even, like certain attractive lid-glands (*N. Lowii, N. laevis, N. Pervillei*), they are so encircled and closed in by the epidermal covering that the gland becomes 'perithecioid' (Pl. XX, Figs. 16, 17), and the sugary secretion exudes from a small circular orifice of the epidermis. On tendrils and on the under surface of the lamina the perithecioid form is characteristic, and it attains its most gigantic proportions on the tendril of *N. bicalcarata*, where, owing to rapid growth of the tendril, the gland-orifice becomes slit-like (Pl. XX, Fig. 14), and the gland-tissue may be one-eighth of an inch in length and one-sixteenth part of an inch in width.

On the stem still greater modification occurs. In Pl. XIX, Fig. 12, a section is represented taken from the stem of *N. Phyllamphora*. Here the gland-tissue has become deeply and sharply involuted; a lumen, spindle-shaped below, constricted above, and again widening, opens by a small

circular orifice; the three layers of gland-cells all show clear finely-granular protoplasm, and a vascular diverticulum ends beneath. But in all cases the diverticulum is separated from the gland-tissue by two layers of bead-like cells, which in position and function seems to correspond to the *membrana propria* of animal glands. The similarity of this to a simple animal gland in shape, structure, excretion, and vascular supply is obvious, and need not further be dwelt on. The resemblance, however, is even more striking in the pedicel gland of *N. bicalcarata*, as illustrated in Fig. 15, where a tendency to branching of the gland-tissue occurs. From each gland a clear viscid juice exudes which is readily sipped by insects, ants and cockroaches exhibiting a special fondness for it in plant-houses. When the juice is left undisturbed for a few days in warm sunshine it solidifies into a white sugary crust, still acceptable to insect guests, who crush and munch it with evident relish.

I have already noted that while the alluring glands are practically absent on the upper lamina in nearly all species, a few afford a marked exception. These are *N. sanguinea*, *N. Northiana*, and to a less degree *N. bicalcarata*. The first two of these have soft, rich, green leaves, and on their upper surface as many as twenty to fifty glands may be counted. From their direct exposure to the sun's rays the secretion soon dries, and in time appears as a coiled white thread from being constantly and steadily added to, as fresh material is poured out. Dried coils three-eighths of an inch in length have been seen protruding from orifices.

As to the average number on any leaf, the following statistics will give an approximate estimate. In *N. ampullaria* three leaves showed respectively 35, 50, and 41 on the under side, and 5, 7, 10 on the outer pitcher-surface. In *N. Rafflesiana*, three leaves showed respectively 8, 15, 11 on the former, and one pitcher had as many as 50 on the latter. In *N. Veitchii* three leaves showed 70, 64, 81 on the former; 115, 83, 121 on the latter. In *N. sanguinea* three leaves showed 65, 91, and 78 on the under side of the leaf; 49, 64,

and 53 on the upper side of the leaf; while a pitcher had 82 over its exterior. In *N. Phyllamphora* there were 45, 28, 24 on the under side, and 18, 29, 34 on the pitcher exterior. In *N. khasyana* there were 45, 76, and 61 on the under leaf side, 108 on the dorsal area of the pitcher between the wings, and 152 over the ventral part, or a total over the pitcher exterior of 260. In *N. Mastersiana*, a hybrid of the last, a fine pitcher showed a total of 266. It will thus be seen that the amount varies greatly, but as a rule the more highly evolved forms have the greatest number over the external pitcher-surface. The tendril of some species was entirely destitute of them, while others were richly provided. *N. bicalcarata*, for number and individual size of the glands, is pre-eminent.

Attractive lid-glands. Little remains to be added regarding these. Usually flat and exposed, they may have a downward-growing epidermal flap, more or less covering them, which ensures retention of their secretion in the cavity thus produced or trickling of it towards the pitcher-mouth. But in several species a perithecioid modification may occur. Thus Dickson pointed out that *N. laevis* (*N. Teysmanniana*), which has alluring perithecioid glands, shows these on the lid also, and this alone can distinguish the species from *N. gracilis*, with which it is often confused. In the aberrant species *N. Pervillei* the gland-tissue is deeply sunk in the lid-substance, and the secretion oozes out by an extremely minute orifice (Plate XX, Fig. 17). But *N. Lowii* furnishes us with the most striking condition. Opening by relatively small orifices among the bristles that beset the large lid are huge perithecioid glands often one-sixteenth of an inch in diameter. As seen in Plate XX, Fig. 16, the secreting layer is raised into several folds, and one might expect that the excreted material would be correspondingly abundant. I have never had an opportunity of seeing this species alive, being indebted to Messrs. Burbidge and Veitch for my specimens, but the former states from personal observation that the liquid exuded is copious. The honeyed juice of all is readily sipped by insects.

Attractive marginal glands[1]. These glands, which appear in the first foliage-leaf of seedlings, and which are the first indicated in developing adult leaves, I regard as the most constant of all. Attention was first drawn to them by Dr. J. Gibbons Hunt, of Philadelphia[2], who stated that 'in *N. Rafflesiana*, *N. distillatoria*, and *N. Phyllamphora*, and probably in all the species, are large cylindrical glands which pour out their secretion through *distinct excretory ducts*. . . A dense tissue of cells surrounds and thoroughly embeds these glands in *Nepenthes*, and this peculiarity of position renders excretory ducts necessary for the secretion to find its way into the pitchers.' In 1883 Dickson published[3] observations on their structure and the relation of the glands in adult and seedling forms.

For isolation and examination of the marginal glands I have found that a very quick and efficient means is by macerating the pitchers in boiling potash-solution for fifteen to thirty minutes, according to the age and consistency of the pitcher, and then, after washing in water, to cut off the collar. The inner epidermis of a portion is then gently pulled up till near its junction with the marginal glands, and after water has been dropped on the slide that carries the object, all the mesophyll-tissue is removed by forceps, along with the vascular bundles. It is then carefully washed and the inner epidermis pulled off, while, if the glands tend to rise or separate with it, a sharp-edged scalpel is softly laid across them to keep them against the epidermis of the collar now remaining. Preparations thus made of *N. ampullaria* or *N. Hookeri* are of extreme beauty, and show (Plate XX, Fig. 21) the glands lying in a row, each in line with its orifice. In the majority of species their size varies strikingly with the relative depth of the conducting

[1] I had hoped that an exhaustive account of these would have been prepared by the late Professor Dickson, partly from many preparations that he had gathered partly from additions which I was privileged to make in the course of these inquiries, but we now mourn his early removal from us. Dr. Archibald Dickson, of Hartree, has very kindly placed all the material at my disposal, and from this, supplemented by my own slides and notes, I draw the following account.

[2] Proc. Phil. Acad. Nat. Science, 1874.

[3] Gard. Chron. (n. s.), Vol. xx, 1883.

surface and elongation of the pitcher, being least in *N. ampullaria* and largest in *N. Edwardsiana*, where they may be $\frac{3}{8}$ inch in length and $\frac{1}{15}-\frac{1}{10}$ inch in width. But in the group of forms represented by *N. gracilis*, *N. zeylanica* (Plate XX, Fig. 26), &c., with narrow incurved collar, they are very small, and in *N. laevis* they are only $\frac{1}{80}-\frac{1}{70}$ of an inch long.

Beside descriptions given in Professor Dickson's communication, I may add the following which I have since gathered. Those of *N. echinostoma* are inserted in a depression at the extremity of each isolated tooth of the collar and are oval in shape (Plate XX, Fig. 24). In *N. Lowii* their position is beautifully indicated in the plate that accompanies Sir Joseph Hooker's description of the species[1]. Round the simple margin minute apertures appear in the middle of little papillae; each leads into a cavity, the gland of which is elliptic and blunt at the extremity.

The position and relation of each gland to the surrounding tissue is illustrated in longitudinal view in Fig. 22 of Plate XX, and a transverse section in Fig. 23. In the latter the presence of two layers of clear oval or almost circular cells immediately outside the gland-tissue proper and internal to the circle of bundles can readily be demonstrated, and traces of them can be followed with some difficulty in longitudinal view as elongated spindle-shaped cells. These correspond to the similarly related cells of the alluring glands already referred to, and those of the peptic glands next to be studied.

A comparative view of four glands drawn to scale from widely distinct species is shown in Figs. 25–28, while their position and appearance in a seedling pitcher from the seventh leaf above the cotyledons is illustrated in Fig. 29. One seldom *sees* a copious secretion from these, but the difficulty of throwing full light on the shaded cavities may explain this. That the secretion is intensely liked by ants and cockroaches I have proved by repeated observations.

Digestive glands. These, supposed hitherto to be associated with the secretion of a digestive substance, are arranged over

[1] Trans. Linn. Soc., Vol. xxii.

the whole interior of each pitcher in such types as *N. ampullaria*, *N. Hookeri*, *N. Lowii*, *N. Rajah*, &c., and in such instances the glands next the orifice are small, but become gradually larger towards the pitcher-bottom. Thus in the most exaggerated case, that of *N. Lowii*, the digestive glands nearest the orifice of the pitcher are $\frac{1}{60}$ inch in diameter in a large pitcher, but the shagreen-like areas formed by each below may be $\frac{1}{16}$th of an inch in diameter (Pl. XXI, Fig. 35). As elongation of the pitcher and deepening of the conducting surface proceeds in the different species, they get restricted to the lower ventricose part. The size and number of cells composing each gland, the number of glands in a given area, and the size of the covering flap, may vary greatly; and even in a single pitcher considerable variations may be observed. Thus in a large pitcher of *N. Lowii* the upper glands are of extremely small size, widely apart, and completely covered by a deep flap. Easy transitions can be traced till we reach the bottom, which is paved with huge glands stuck closely together, and separated from each other by ridges of thick-walled cells (Fig. 35), which above may show a faint trace of a flap. Again, in such widely different forms as *N. Rajah* and *N. albo-marginata* there is a similar condition of things, though less pronounced. But in the two outlying species that are widely separated geographically, viz. *N. Vieillardii* of New Caledonia and *N. Pervillei* of the Seychelles, we have each gland sunk in a deep depression of the epidermis, which forms a bag-like covering with narrowed mouth directed downwards (Pl. XXI, Fig. 34). The latter species is the most exaggerated. In *N. Lowii* the upper glands, though widely apart, amount in a square inch to about 2,000, the very large densely-packed ones of the pitcher-bottom vary from 250–600 in a square inch. In *N. bicalcarata* the round button-like densely-set glands amount to from 5,000–7,000 in a square inch. Hooker gives for *N. Rafflesiana* 3,000, but even more may be counted in some pitchers of that species. In all cases their structure agrees with that of the alluring or attractive lid-glands.

Development of the glands. The development of the glands on the detentive surface has been already studied by Wunschmann[1], and with slight additions the description can apply to all except the large marginal glands. The development of those lid-glands the surface of which is flat or nearly so, and which are slightly sunk in pocket-fossae, agrees exactly with that given by him. In the formation of the alluring perithecioid glands of leaves, and in similarly shaped glands of some lids, an evident epidermal depression in the region of the future gland appears about the time that division in its cells is beginning. Owing partly to rapid division and growth of the marginal gland-cells, but specially to similar activity in the surrounding epidermal cells, these last rise up round the central gland-mass, and cover it in until only a small circular or elliptic aperture is left in the middle of the covering-in cells.

Each marginal gland is first indicated by the simultaneous depression of the marginal epidermis and out-bulging of cells at the bottom of the depression in obovate outline. As in all the other glands, division of the epidermal cells into three layers —an outer one of columnar elements and two subjacent ones of polygonal elements—occurs, while, so far as I can trace, a few are cut off from the innermost of the three to form the rudiment of the pulp-cells of the gland. This condition is represented in Pl. XXI, Fig. 30, and is permanently retained in the first six to eight seedling leaves. Comparison of Fig. 30 with Fig. 29, taken from the seventh leaf-pitcher of a seedling, shows that in both each gland is obovate in outline and protrudes slightly from the depression. But in the former, by continued outgrowth of the walls of the depression and sinking of the gland into the cavity, it eventually occupies the deep and hidden position which caused them to be overlooked by most observers.

Professor Dickson referred to the usual presence in large marginal glands like those of *N. khasyana* and *N. Phyllamphora*, of a central cavity traversing the length of each, into which elongated cells of the gland projected. These appeared

[1] Op. cit. pp. 17, 18.

to be so invariable in certain species as to suggest that the cavity resulted from epidermal involution, but it is evidently due entirely to disruption of the central secreting cells whose origin has been traced above.

The opportunities offered by the variety of *Nepenthes* cultivated at the Royal Botanic Garden, Edinburgh, for observing the behaviour of insects have been often taken advantage of by me. The warm houses in which they grow are constantly infested by colonies of a small brown ant about half the size of our fallow-ant. These make nests of from one to three inches across in the pots and frame-baskets carrying the plants. On a warm day they are constantly running about, and a large share of their attention is given to the growing specimens. In one of the houses cockroaches are abundant, and the pitchers of *Nepenthes* are favourites with them during night. I may now describe an observation made in the summer of 1885. Being in the house just indicated at 8.30 p.m. on a clear evening in June, a large cockroach was noticed to be perched on the front part of the corrugated collar of a fine pitcher of *N. khasyana*. Approaching cautiously, it was seen to bend its head into the pitcher-cavity and sweep it rapidly in successive jerks round the inner edge of the corrugated collar where the products of the marginal glands would lie. Sipping the material from these, it then rested for a moment and enjoyed with evident relish the cleaning of what adhered to its mandibles. It then repeatedly tried with its fore-legs to step on to the conducting surface of the pitcher-cavity, but always slipped, so leaving this it reared itself on its long hind-legs by planting one on each side of the rim, catching with the middle legs on to the lower sides of the lid. Placing its fore-legs on the middle of the lid, it swept with its mouth-parts the richly honeyed surface in long lines. But this did not appear to be satisfying, when compared with the product of the marginal glands, for it speedily returned to these and renewed its jerking mode of feeding. It again attempted to get into the pitcher-cavity, but finding this unsafe, it finally licked up traces of the marginal gland-secretion which its fore-feet had smeared on the cor-

rugated collar. Running down the outside of the pitcher it passed up the tendril and on to the under laminar surface, where its presence would have been perfectly unsuspected had any insectivorous birds been in the neighbourhood, and where also, as Mr. Symington Grieve has suggested, it would have been sheltered from the sun's heat in the daytime. I gently scratched the upper surface above where it was, and it at once retraced its steps in a hurried manner, till it reached the outer surface of the pitcher. Here it rested for a time sipping the juice which exuded from alluring glands, but it soon passed to its old position on the collar. Though disturbed a few minutes before, it seemed quite to forget its fright, and again fell to cleaning the marginal gland-orifices with the utmost care and gusto. It rested now and again only to resume operations, once making a short excursion to the lid-surface, which appeared to me to offer far greater attraction, but seemingly regarding this as inferior it returned to the collar. Constantly trying to get on to the conducting surface and as often foiled, it again ran up along the tendril to the under side of the lamina. Again I scratched this, and the former course was taken, the former efforts made. I was greatly struck by the careful way in which, while attempting to pass into the pitcher, it hooked its two strong hind-legs over the reflexed collar-margin, and by the ability it showed to pull itself back by these alone, the second as well as the first pair of legs often being inside the pitcher. Tired of its movements after the fifth excursion, and finding that twilight was approaching, I finally jerked it into the cavity with my pencil, as it hung on the ridge exploring the interior. In its fall it quickly spread out its long legs against the sides of the conducting surface and struggled violently to get out. For a short time this proved useless—it rather slipped deeper; but after one severe effort, it hooked the claws of its fore-legs over the corrugated rim and pulled itself out. I considered that it had fairly earned liberty and it speedily moved off. Before leaving I looked into the cavity and saw two decaying cockroaches in the bottom. Returning next morning with Professor Dickson

and Mr. Lindsay to show them the pitcher, a living one of the size that had been watched was inextricably struggling in the bottom. Whether it was the observed one of the previous evening could not be determined. This was the first opportunity that presented itself of proving that the marginal gland-secretion is exceptionally attractive, but to further verify this I have since watched ants for hours. The results confirm and amplify what has already been said. Running up the stem the insects turn to right and left in quest of food; a globular drop oozed out from an alluring stem-gland arrests them for a time; at this they sip and tear, the secretion being viscid, or often dried into a white sugar-like substance. Leaving this they pass on to the leaf-base, and almost invariably keep its under side. This, as already stated, appears to be a device to shade them from enemies and warm sun. Not only so, in many species the alluring laminar glands are greatly massed along the sides of the thick mid-rib, and the shadow on one side of it affords further protection. Moving on restlessly and sipping as they go, they reach the tendril, which in some species, notably *N. bicalcarata*, offers a rich feast. The wing-like pitcher-flaps and areas between are more beset with alluring glands than the rest of the exterior, and along this therefore, in most cases, they pass till they come to the orifice. The attractive lid-glands prove in most species, particularly in *N. sanguinea*, *N. khasyana*, and *N. Phyllamphora*, a great attraction, but even these sink into insignificance if the insect reaches the marginal glands. Straining to get at the orifices of the glands, they over-reach, and, falling into the cavity, in very rare cases indeed, out of the dozens that I have watched, is escape possible. The irregular and struggling efforts made by insects on the conducting surface of a pitcher is highly instructive, and demonstrates how extremely effective it is for the work in hand.

It will thus be seen that running insects frequent *Nepenthes* in our conservatories, unlike the Sarracenioids, which, as already stated, are practically only visited by flying ones. It is not that the latter are excluded, for on warm days, when the top

ventilators are open in the Sarracenia- and Nepenthes-houses, they pass into the former, but seem to avoid the latter. It may be a question of relative heat with the insects, but the matter can only be satisfactorily settled by examination of pitchers in their native haunts, or of the contents of such if carefully transported to this country.

The number of insects caught is frequently very great, and some species seem to excel in this: *N. Hookeri*, *N. Rafflesiana*, *N. albo-marginata*, *N. khasyana*, *and N. Phyllamphora* are the finest catchers; *N. gracilis*, *N. sanguinea*, *N. Teysmanniana*, and *N. distillatoria* are indifferent; while *N. ampullaria* is bad. I give this only as my experience in plant-houses, but when wild the results may be different, though I do not think widely so. That the presence of insects in the cavities either by sight or odour helps to attract others has often occurred to me as being an aid to the honeyed bait. That they draw the higher animals is undoubted, and I may here notice the ingenious and likely hypothesis which Mr. Burbidge has proposed to account for the two spurs of *N. bicalcarata*. The rodent *Tarsius*, in the region where the plant grows, frequents various *Nepenthes* to rifle the pitchers of their insect earnings by bending down and shovelling out the contents. It has learned, from being caught in the nape of the neck by the two spurs, to shun the species named. Four seasons ago, a rather small pitcher of *N. Hookeri* caught within a fortnight seventy-three cockroaches, large and small, having been emptied out on three occasions. The odour resulting from digestive decomposition would undoubtedly tempt those succeeding the first few caught. My experiments have not been extended enough to enable me as yet to advance our knowledge of the digestive action carried on in the pitchers, but I hope to publish on this at a later period.

I can scarcely leave this part of my paper without referring to the remarkable relation which *N. bicalcarata* has to an ant that frequents it, as described by Mr. Burbidge. The matter completely puzzled me, till that gentleman kindly gave me his simple but original explanation. In seven out of every

ten pitchers which one may examine from their native haunts, a neat round hole is drilled in a swollen region of the tendril opposite the pitcher-bottom, while all pitchers, whether brought from their natural habitat or grown in conservatories at home, show the fusiform swelling. The explanation seems to be that this kind of ant, finding it could not reach the cool juice of the pitcher-cavity in the ordinary way without permanent risk to itself, has learned, on the physical principle that water will rise to its own level, to drill a hole in the tendril, and the liquid filtering up through the cells oozes out to regale the waiting ant. But the most interesting point about the condition is, that while plants grown in our conservatories are not troubled by the insect, the continued hypertrophy brought about by the constant liquid supply has so affected the constitution of the plant that the acquired character is hereditarily transmitted. Mr. Burbidge has informed me that the centre of the swelling is generally hollow, but both in material brought home by him and kindly placed at my disposal by the Director of the Royal Gardens, Kew, as well as in home-grown pitchers, if these are rather young, the tissue is solid though loose in texture, a breaking down of the cells only taking place in the older specimens.

VI. General Morphology and Histology of the Flowers of Nepenthes.

I have already stated (p. 416) that the Sarraceniaceae show many points of affinity in flower-structure with the Nepenthaceae, and the affinity in pitcher-morphology has been already explained. Though somewhat of a digression, I may be allowed to discuss the systematic characters of the two orders, for I feel convinced that we have to deal with a group of plants that constitute a very natural alliance, though, owing to wide isolation in past ages, they have diverged in points which are of generic importance only. For comparison I subjoin the systematic characters of both orders in parallel columns.

Sarraceniaceae.	*Nepenthaceae.*
1. Perennial herbs with rhizomes, growing in marshes.	1. Under-shrubs with feeble stems, growing in marshes and wet places.
2. Leaves with expanded sheathing leaf-base, ascidiform by excavation of the mid-rib, glandular and hairy without and within, or within only.	2. Leaves with expanded leaf-base, ascidiform by excavation of the mid-rib, glandular and hairy or glabrous without, glandular within.
3. Leaf-glands multicellular, alluring attractive (and digestive?) in function; seldom or never connected with bundles.	3. Leaf-glands multicellular, alluring attractive (and digestive?) in function; of large size, and connected with bundles.
4. Pitcher continuous with the basal leaf-portion.	4. Pitcher continuous with leaf-base in seedling, but separated in the adult by the intervention of a tendril.
5. Inflorescence solitary or racemose; flowers hermaphrodite, large, and green; greenish-yellow, yellow-red, or reddish purple.	5. Inflorescence racemose, flowers small, dioecious, rarely hermaphrodite in teratological specimens; green, greenish-yellow, or red.
6. Sepals 4 or 5, green or slightly petaloid, covered with glands like the attractive lid-glands, glabrous, free, hypogynous.	6. Sepals 4, rarely 3, green or slightly petaloid, covered with glands like the attractive lid-glands, hairy without, glabrous and glandular within, free or slightly connate, hypogynous.
7. Petals 0 or 5; large, free, hypogynous.	7. Petals, 0.
8. Stamens indefinite, free, hypogynous; anthers 2-celled.	8. Stamens (of staminal fl.) 4–16 connate, hypogynous, anthers 2-celled.

Sarraceniaceae.	*Nepenthaceae.*
9. Ovary free, 5 3 celled; placentas attached to the inflexed margins of the septa.	9. Ovary free, 4–3 celled; placentas septal.
10. Style short, with simple, or truncate, or dilated extremity, bearing 5–3 stigmatic lobes.	10. Style short, with simple truncate extremity and bearing 4–3 stigmatic lobes.
11. Ovules numerous, multiseriate, anatropal.	11. Ovules numerous, multiseriate, anatropal.
12. Fruit a 5–3 celled capsule, dehiscing loculicidally, and surrounded by the persistent calyx.	12. Fruit a 4–3 celled capsule, dehiscing loculicidally, and surrounded by the persistent calyx.
13. Seeds indefinite, small, ovoid and wingless with crustaceous testa, or elongated with loosely reticulate testa, or ovoid with winged testa.	13. Seeds indefinite, small, ovoid and wingless, or elongated and with loose reticulated wing-like testa.
14. Albumen copious, fleshy.	14. Albumen copious, fleshy.
15. Embryo ovoid or cylindrical, straight in the albumen.	15. Embryo sub-cylindric, straight in the albumen.

The agreement of the above characters establishes not a mere analogy, or remote relationship between two orders, as botanists have hitherto supposed, but gives to all the genera a common ordinal value; for the herbaceous or semi-shrubby mode of growth, presence or absence of tracheids in the tissues and of petals in the flowers, the distinct or fused stamens, and other points of difference, are not sufficient, and have not been made sufficient by systematists hitherto, for the separation of genera that otherwise showed decided affinities.

The following condensed description would enable us to diagnose readily from all others an order which might appropriately be termed the Ascidiaceae:—

Glandular herbs or under-shrubs, inhabiting swamps and

wet places; stem creeping prostrate, or upright and ascending by tendrils; leaves with or without flattened lamina, sometimes tendriliform, ascidiform, and adapted for insect-catching; flowers regular, solitary, or racemose, hermaphrodite or dioecious, entomophilous; sepals, 5, 4, *or* 3, *green or coloured, hypogynous; petals,* 5, 4, *or* 0, *greenish or coloured when present; stamens indefinite (rarely definite), hypogynous, free or connate into a tube, anthers* 2*-celled; pistil syncarpous, of* 5, 4, *or* 3 *carpels, with central (axile) placentation; ovules anatropous, horizontal or ascending; style short, with truncate flattened or expanded extremity, bearing* 5, 4, *or* 3 *stigmatic lobes; fruit a capsule, surrounded by the persistent calyx, dehiscing loculicidally into* 5, 4, *or* 3 *lobes when ripe; seeds small, ovoid, elongate-appendiculate or winged, testa crustaceous or loosely reticulate; albumen fleshy; embryo straight in the albumen.*

I have been able to examine minutely the flowers of most of the Nepenthes, and in all the under-surface of the sepals is clothed with fine hairs like those on some parts of the foliage-leaf, particularly the pitcher. Lying amongst these may be a few perithecioid glands which in *N. bicalcarata* resemble those of the under surface of the lamina and are equal in size or larger. From 3–6 may occur on each sepal, but they are absent or very scarce in most of the species.

Of special interest is the fact that in *N. Pervillei*, greatly isolated geographically and modified morphologically, the flower-stalks as well as the sepals are abundantly glandular. The glands of the stalks are very abundant, small, considerably elongated, and sunk usually in rather deep cavities (Plate XX, Fig. 18). The upper surface of the sepals in every species is closely covered with nectar glands (Plate XX, Figs. 19, 20), which even an expert could not distinguish from the attractive glands of the inner lid-surface. Thus a preparation from the marginal part of the lid and from the upper sepal in *N. khasyana*, when placed under the microscope side by side, would be undistinguishable, except perhaps from the slightly smaller size of the glands in the latter.

There is a decided tendency in many species to sinking and

folding of the gland with restriction of the exposed surface, but in *N. Pervillei* this is carried to such an extent that each gland opens by a very narrow elongated orifice. The shape of the lid-glands, therefore, and those of the sepals is identical. But this does not follow in all cases, for in *N. Lowii*, with huge perithecioid lid-glands (Fig. 16), the sepals have large but open or only slightly constricted orifices.

The amount of nectar secreted is in all cases great. Mr. Burbidge tells me that when flowering the inflorescences are constantly surrounded by clouds of small insects that buzz round them or alight to sip the juice.

The pistil does not call for special note.

VII. Arrangements for Pollination in Flowers of Nepenthes and Cephalotus.

Nepenthes. The flowers being so small and the sepals so inconspicuous individually, one might have expected that wind-pollination would take place: but an entire raceme presents a decidedly striking contrast in the midst of surrounding foliage, and the nectar being copious, any insects attracted are amply rewarded. That pollination is usually effected by aid of the hosts of small insects which hover over the flowers is, as Mr. Burbidge thinks, extremely probable, and the dioecious habit of the plants renders a passage from one to another necessary. But that the outer surface of the sepals in several species and of the flower-stalks in *N. Pervillei*, and occasionally in *N. bicalcarata* and other species, should be honey-baited, suggests the alluring to them in some cases of such running insects as frequent the pitchers, viz. ants, &c.

In our hot-houses these insects visit them in numbers after traversing stems from ten to twenty feet long. Their careful, inquisitive movements, and examination of everything in their path, suggests that they may carry pollen in a locality where *Nepenthes* scramble amongst the undergrowth.

Cephalotus. Professor Dickson drew attention to the extremely diffuse condition of the alluring glands in this genus, which are scattered over the outer surface and stalks of

pitchered leaves as well as of flat unpitchered ones. I find them likewise on the scales of young rhizomes, on the long, slender flower-stalks, and on the bracts which these bear. In the two last cases they occur among the slender, elongated 'encapsulating' hairs of Dickson. On the outer surface of the sepals they are even more numerous and larger.

The receptacular processes between the stamens and carpels are specially curious. Each is a stout, hollow, up-bulging of the epidermis of the receptacle (Plate XXI, Fig. 37), which rarely may bifurcate, but in all cases ends in a flat top, composed of an outer circle of cells with two central semi-lunar cells, showing what is apparently a stomatic orifice between them. I have tried to learn by study of living flowers what these secrete, but have got no satisfactory result. They may exude something to tempt insects amongst the stamens and carpels for pollination purposes, but their appearance suggests rather that they are stalked stomata.

Insects are seldom caught by the pitchers of this genus so far as I know them, and I have never seen insects about the flowers. From the accounts of those who have examined the plant in a wild state, we learn that the pitchers do catch a tolerable number of insects, and that these will be attracted to the flowers is natural, since alluring glands are everywhere very abundant.

VIII. On Hybridity and Relation of the Species to each other in the Different Genera.

In the course of the present inquiry I was greatly impressed with the number and beauty of hybrids obtained by gardeners during the short time that these plants have been in general cultivation. Equally was I impressed, from conversations with well-known importers and cultivators, by the difficulty experienced in deciding whether certain forms raised from wild seed should be viewed as true species or hybrids. I determined, therefore, to include hybrids in the range of my work.

In his article on *Nepenthes* in the Gardener's Chronicle[1],

[1] Op. cit. Vol. xx, 1883.

Professor Dickson gave the known genealogy of some seedlings raised in the Edinburgh Botanic Garden, and now known as *N. edinensis*. A glance at the genealogy will show that species of *Nepenthes* hybridize freely, that hybrids are themselves fertile and by renewed crossing may give rise to offspring which in their turn are fertile. A practical knowledge of the species and hybrids therein named, as well as of others in the market, enables one further to say that so far as habit, shape, colour, and power of propagation go, the offspring shows qualities strikingly characteristic of both parents, and the same equally holds true regarding Sarracenias.

Now amongst biologists it has been asserted, and by none more strongly than the advanced evolutionists, that new forms are produced, not by inter-crossing of species, but by perpetuation of variations in the same species. The objection has often been urged against evolution as ordinarily defined, that its methods are too slow for the results attained in the limited time which phÿsicists will alone give. Can any explanation be given which would account for a hastening of the process? Here we may have what is desiderated, for though hybridization means, at first sight, merely the blending of peculiarities and advantages already gained, and therefore the possession of a form in no way more highly evolved, on closer inspection we can convince ourselves that a real and great advance may be effected. To take a concrete example, *N. sanguinea* is a species whose rich crimson finely-shaped pitchers give it a beauty all its own, while its copious secretion of honey renders it attractive to insects. Its large, soft, succulent leaves and shoots suggest powerful assimilating capabilities. But placed against this is the experience of all cultivators that it does not pitcher freely. On the other hand, *N. khasyana* has long lurid-green and rather unshapely pitchers, its honey-baits are abundant, its leaves are shorter and much narrower than in the previous one, it pitchers freely, and can readily be propagated. As a result of cross-pollination we get the magnificent *N. Mastersiana*, by far the finest of the many interesting hybrids which the Messrs. Veitch have sent out, and which

combines in a remarkable manner the good points of both parents, and few or none of their bad ones. Further, be it observed, the inheritance of these in a single form gives it an advantage over both parents which will go far to perpetuate it, and if the sexual organs are not specially weakened, the superiority will be complete.

Now what the gardener has done artificially in the above case nature does often accomplish unaided. If, then, this holds true of *Nepenthes* and *Sarracenia*, it is highly probable that the results have a wider application. It must be freely acknowledged that the balance of evidence as furnished by the experiments of Kölreuter, Gärtner, Wichura, and Darwin, is opposed to the above, but many factors have to be accounted for before a final conclusion can be reached.

One very important outcome, however, of the microscopic study of hybrid pitcher-plants has been the demonstration that all the cells of these exhibit the peculiarities of both parents in blended fashion. It is not intended in this paper that these should be exhaustively studied; we would merely refer in detail to the structural conditions presented by the interior of the tubes of a hybrid *Sarracenia* and those of its parents.

Amongst a set of seventeen hybrid Sarracenias kindly sent me by the Curator of Glasnevin Botanic Garden, was one now known as *S. Swaniana*, the product of a cross between *S. purpurea* and *S. variolaris*, the latter being the male parent.

Tube-structure of S. Swaniana and of its parents. The surface-cells of the inner lid-epidermis, in parents and hybrid alike, are very wavy in outline. In *S. purpurea* 5–6 stomata can be seen in the field of No. 7 Leitz objective with No. 1 eye-piece; in *S. Swaniana* there are 3–4; and in *S. variolaris* 2–3. The downward-growing hairs (Plate XIX, Fig. 6) of *S. purpurea* are long, stout, and with evident striae, there being 15–16 commonly shown on surface-focussing of a hair. The hairs spring from greatly enlarged epidermal cells, and are mostly uniform in size, one in five only being rather reduced. Seven to eight appear in the field of No. 3 Leitz objective

with No. 1 eye-piece. In *S. variolaris* the hairs are dagger-shaped; each springs by a greatly enlarged base from an epidermal cell, and then rather suddenly narrowing, tapers into a fine point. The surface is finely striate, 25–30 striae appearing on surface-focussing. In size the hairs vary greatly, though all are much smaller than those of *S. purpurea*, there being 35–38 hairs visible at once under the same field as above noted. Similar examination of *S. Swaniana* shows 3–4 hairs under field of view exactly like those of *S. purpurea*, and 15–17 like those of *S. variolaris*, though both are rather feebler in form. The conducting surface in *S. purpurea* is a narrow zone about three-eighths of an inch deep, that of *S. Swaniana* is one to one and a half inches, while that of *S. variolaris* is one and a half to two inches, according to the size of the specimens. In *S. purpurea* each cell of the conducting surface is irregularly quadrangular or pentagonal in shape, and its lower edge is raised into a papilla, towards which a few converging striae unite (Plate XIX, Fig. 6). In *S. variolaris* each of the epidermal cells nearest the attractive surface is rounded-angular in shape, and its lower part is prolonged into a triangular hair-process measuring 20 μ long, while its surface is traversed by fine and dense striae. The corresponding hair-processes that grow out from the lower part of the conducting surface are greatly finer and more tapered, and are twice as long. In the hybrid the cells nearest the attractive surface are rounded-angular, though the angularity is more pronounced than in the last. Each cell has a tapered—scarcely triangular—hair-process from 12–15 μ long, which is traversed by striae, intermediate in fineness and number, between those of the parents. Deeper down they show the effect of the male parent, in that the hairs elongate till they are 25–30 μ long, while occasional cells may have processes 30–35 μ in length, thus indicating a greater unisexual luxuriance in these.

The glandular region of *S. purpurea* is tolerably deep, quite glabrous, and its cells are equiradiate or transversely elongated with extremely wavy walls. The glands are abundant, 3–4

being visible under Leitz objective with No. 1 eye-piece. The demarcation of the glandular and detentive surfaces is very sharp, as the cells of the latter are straight-walled. From a few of these the long slender hairs of the detentive surface (Fig. 6 *d'*) spring.

A separate glandular surface does not exist in *S. variolaris*, and the junction of conducting and detentive surfaces, though sharply marked so far as the size and distribution of the hairs goes, is scarcely traceable in the character of the surface-cells, which remain nearly uniform throughout. In the hybrid one reaches an area just below the inferior limit of the conducting surface, where many of the epidermal cells are devoid of hair-processes, are considerably elongated, and have sinuous walls, thus presenting a very average type between the wavy-walled cells of the one parent and the straight-walled cells of the other. But a few of the cells are thick and straight-walled, and grow out into long delicate hairs typical of a detentive surface. It is the occurrence of these on what is undoubtedly the representative in the hybrid of the glandular surface of *S. purpurea* that causes me to regard that surface as a modification of the upper part of the detentive area. There are numerous glands, as one might expect.

The surface-cells of the detentive region in *S. purpurea* are polygonal and rather thin-walled; the detentive hairs are very long and fine (Fig. 6 *d'*), the larger ones measuring 1·6 mm. Glands are entirely absent. The surface-cells in *S. variolaris* are elongate-sinuous and thick-walled; the detentive hairs are fine but short, the longer ones measuring ·5 mm. Numerous glands are found over the upper region, but disappear entirely from the lower. In the hybrid the surface-cells are slightly elongate and show faint traces of a sinuous outline; in thickness they rather seem to approach the latter parent; the longest detentive hairs are 1 to 1·2 mm. It is thus evident that every epidermal cell of the hybrid pitcher reveals the blended action of the sexual elements of both parents. As regards the lid-hairs and the cells from which they spring, one might suppose that these are reproduced in an unaltered

manner, but the change observable in their size as compared with those of the parents proves that they equally are altered.

Every species of *Sarracenia* has now been crossed, and many of the hybrids have been successfully recrossed. The same is true of cultivated species of *Nepenthes*, and it remains to be seen how far these will show fertility and keep true to the inherited features.

IX. Supplementary Note on the Morphology of the Leaves and Pitchers.

In No. 13, Vol. iv of the Annals of Botany, Professor Bower has subjected to detailed criticism and comparison his own and my views regarding the morphology of pitchered insectivorous Plants, and since the latter part of my paper was in MSS. Professor Goebel [1] has expanded his previously expressed opinions [2] on the subject. A few words in reply may not be out of place.

Both writers, I believe, err in tracing the earlier developmental stages, without attempting to connect these step by step with all the peculiarities shown in the mature condition. Thus Professor Bower objects to the view that there is more than one pair of leaflets in *Nepenthes*, and this at once leads up to the fundamental difference in our treatment of the subject. He regards as leaflets only such rounded outgrowths of the mid-rib as develop early from it in an isolated manner, and by comparatively narrow attachment. Such a limitation would involve the assumption that leaflets are formations distinct from, and at no period in their history derived from, the lamina or the 'wings of the phyllopodium.' But developmental evidence and the structure of mature leaves alike prove that they are lobes of the 'wings' which by localized and intercalary growth have been separated from the latter. The transitional stages in the process furnished by Umbelli-

[1] Pflanzenbiologische Schilderungen, Part II, 1891.

[2] Vergl. Entwickelungsgeschichte der Pflanzenorgane, Schenck's Handbuch der Botanik, Bd. iii.

ferous, Acanthaceous, and even more ancient alliances like the Ferns, are too numerous to admit of doubt. Complete verification of the position is furnished by leaves of *Gleditschia triacanthos*. In addition to the ordinary pinnate leaves that appear chiefly on shoots from second year's wood, and the bipinnate, more rarely pinnate, leaves that spring from the first year's wood, hundreds of examples can be got from a single tree, where every transition-stage, from an entire leaflet to one cut up into secondary leaflets, is presented. Some of these are illustrated in Figs. 38–43, and clearly prove that leaflets, historically, are restricted parts of an originally continuous lamina or 'wings of the phyllopodium.' Localized growth of one region is usually associated with intercalary or apical growth of another, and thus leaf-indentations, leaf-lobes with broad insertion on the mid-rib, and leaf-lobes with narrow insertion (so-called 'pinnae') are transitional phases in leaf-modification. To fix on any one of these in its earlier development, and arbitrarily separate it from the others, no matter what the subsequent history is, can only obscure the true issues. On this account I would designate as leaflets any portions of the originally continuous lamina, or 'wings,' which become completely isolated along the mid-rib at any period of development up to the stage when maturity is reached. Certainly it might be convenient for descriptive purposes to use terms, as is actually done, that would approximately indicate stages in leaf-division, but at best these would be inconstant, and conveniences of expression only.

It is impossible moreover to restrict the term leaflet to 'such growths as arise at an early period in definite order upon the wings,' for in the leaflets of *Gleditschia* already cited, as well as in many other plants, such order is often broken through.

Goebel, speaking of the pitcher-flaps in *Nepenthes* and *Sarracenia*[1], says, 'in allen Fällen sind diese Flügel nachträglich entstandene Wucherungen,' but such is not the case,

[1] Op. cit. p. 102.

as Goebel's figures prove. They are traceable in the four genera now reviewed as early as, or soon after, indications of the pitcher-invagination appear; and in *Nepenthes* Goebel acknowledges that what is an originally continuous leaf-blade in earlier development, becomes by intercalary growth separated into a pair of pitcher wings and basal wings (p. 103). He takes exception to these being termed leaflets, but in view of what has been said above I must adhere to the explanation given. Bower objects to the lateral flaps of a *Nepenthes*-pitcher being viewed as leaflets since they originally form parts of two 'smooth flanges in very early stages of development.' Not merely the early stages of development, but all succeeding ones are of value, and that these flaps should become isolated distally from the proximal part of the flanges is proof to me that while the lid-leaflets of *Nepenthes* are now early and sharply isolated from the flanges, the pitcher-flaps retain connexion for a longer period and by a broader base. This view is quite supported by the fact that in *N. Rajah*, *N. Curtisii*, &c., the basal parts form a distal peltation demarcating them, even if the long tendril did not, from the pitcher-flaps, which themselves, in all pitchers that I have examined, exhibit similar distal peltation in front of the orifice, as do the lid-leaflets throughout their entire history.

It is not necessary therefore for me 'to show that distinct rounded outgrowths do appear on the wings of the young leaf'; all that is required is, what actually occurs, viz. the localized outgrowth on the front of the pitcher of portions that are ultimately quite distinct from those of the base.

No attempt is made by Goebel to explain the two swellings in *Nepenthes* that arise in line with the laminar flanges, and which unite to form the lid, though these exactly correspond to the lobes that he figures (Pl. XIX, Fig. 6) on the developing leaf of *Darlingtonia*. In a footnote (p. 102) he says, that 'nicht jede Einbuchtung an einem Blattrande als Anlage einer Gliederung des Blattes betrachtet werden kann. Es wäre dazu, da die Entwicklungsgeschichte für *Nepenthes*

selbst zu einer solchen Auffassung keinen genügenden Anhaltspunkt bietet, die Kenntnis einer verwandten Form mit gegliederten Blättern notwendig.' To the writer this appears a very loose method of discussing or explaining a morphological feature. In his description to which he refers in Schenck's Handbuch (III. i, p. 238), he says, 'Der Deckel is nur das obere Ende der Blattlamina.' If the lid is only the upper end of the leaf-blade why does it usually show indications of two separate lobes, why are two distinct vascular bundles prolonged into it, and why is there a transverse vascular and even laminar connexion of the pitcher-wings in front of and beneath the pitcher-orifice? These problems are left untouched. In all four genera, *Nepenthes*, *Heliamphora*, *Sarracenia*, and *Darlingtonia*, we have to do with a greatly more complicated structure than a peltate leaf, and till it is viewed in a different light we cannot expect a better interpretation than that given by Goebel. Undoubtedly in *N. Rajah*, *N. Northiana*, *N. Curtisii*, &c., we have to deal with at least three successive laminar peltations from base to apex of the leaf, no matter what name we give to the laminar expansions that form these.

Bower practically rejects the conclusion that the lateral growths on the terminal spur of a *Nepenthes*-leaf are rudimentary leaflets. They are lateral outgrowths from the sides of the mid-rib, in line with 'the smooth flanges' and the lid-lobes. They appear as early as, or earlier than, the lid-lobes, and I am unable to see under what possible category they can be placed if not under that given by me.

Objection is also taken to the median flap of *Sarracenia* being regarded as a laminar fusion, and to this view being supported by comparison with the *Iris*-leaf. But in quoting Goebel's evidence on the latter[1], Prof. Bower seems to attach no importance to the longitudinal vascular-bundle-distribution alike in embryonic and mature leaves, which entirely favours the view I advanced. If further evidence were needed, this is furnished by the leaf of *Phormium tenax*, which is widely

[1] Schenck's Handbuch der Botanik, III. i, 219.

expanded in its leaf-sheath, as in *Iris* and *Sarracenia*, then fuses along the middle region, so as in some cases to entirely or partially obliterate the free faces, and again opens out in its terminal portion. Now both in *Iris* and *Phormium* the most posterior vascular bundles that traverse the free faces of the leaf-sheath are prolonged straight up to the organic apex, thus demarcating the wide extent of fusion that has occurred in the upper leaf-faces. In seeking therefore for 'evidence from external form' solely or chiefly, there is the danger, we believe, of the mark being overreached. It is further suggested that I regard the leaf of *Iris* as a compound one. No such statement has ever been made by me, though I feel I was quite justified in comparing the fused laminar faces of *Iris*-leaf with the similarly but more perfectly fused leaflet-faces of *Sarracenia*.

I can see no relation between the phyllode of an *Acacia* and the median flap of *Sarracenia*, since the latter is not a flattened petiole, but a median process growing out from a rounded and well-developed mid-rib. To regard it, particularly in view of its affinity to *Heliamphora*, as a fusion of laminar faces, rather than 'a phyllodineous flap,' appears to be the more simple and natural explanation.

As regards the lids of *Sarracenia* and lips of *Darlingtonia*, I must still view these as leaflets, and Mr. Aldrich Pennock brought me during the past summer an interesting confirmatory specimen in a pitcher of *S. purpurea*. This showed (Fig. 44) a sharp transverse fusion of the vascular bundles across the mouth of the pitcher posteriorly, and a mid-rib prolonged from this and ending in a minute point, the organic leaf-apex. From either side sprang two expanded lobes that were supplied with vascular bundles from the mid-rib. Goebel's beautiful illustrations of *Darlingtonia* confirm this; though we must take exception to the statement that he makes regarding these (p. 84), 'dieselben als Fiederblättchen zu betrachten liegt wohl kein Grund vor. Es ist eben eine Teilung der Blattspitze, welche dieselbe zu einem noch auffallenderen Anhängsel des Schlauches macht, als es der

einfache Lappen der Primärschläuche ist.' Their structural relations and vascular supply prove them to be lateral paired outgrowths from beneath the apex, and therefore confirmatory of the writer's contention.

Professor Bower says: 'Dr. Macfarlane would appear to recognize any convexity of margin of the wing, however slight and however late in its appearance, as a leaflet or pinna, whereas I should reserve these terms for only such growths as arise at an early period in definite order upon the wings and appear as convexities with a clearly defined contour. Pursuing his less rigorous method, Dr. Macfarlane finds himself landed in a view as to the leaves which is too unwieldy to appear natural; my own explanation has at least the quality of relative simplicity.' In the first part of the above quotation, Professor Bower misinterprets me, for as already noted I should limit the term leaflet only to such divisions of the wings as are completely isolated down to the mid-rib, no matter when they appear or how broad their attachment. In this way only has it been used. Rigorous methods are wholly commendable, but arbitrary ones may lead to imperfect views, and such expressions as 'wings of the phyllopodium,' 'developments of those wings,' 'a phyllodineous flap,' and 'a flattened termination of the leaf' result.

In attempting to reduce such pitchered leaves to the standard of simple peltate ones, Goebel has no natural explanation for the dorsal flaps in all the genera, for the successive peltate fusions in *Nepenthes*, or for the lid-formation in all. One reason for my advancing the opinions already given was that it introduced a simple uniformity in explanation of these puzzling types which in effect coincided with results drawn from observation of anatomical detail and floral structure. I must therefore adhere to the views already expressed.

The author gratefully acknowledges his indebtedness for supplies of material to the Directors and Curators of the Royal Botanic Gardens of Kew, Glasnevin, and Edinburgh, as also to Messrs. Burbidge, Courtauld, and Veitch.

DESCRIPTION OF FIGURES IN PLATES XIX, XX, AND XXI.

Illustrating Dr. J. M. Macfarlane's paper on Pitchered Insectivorous Plants.

Plate XIX.

Fig. 1. Vertical section of *Darlingtonia* pitcher, showing gland-cells. In one of the adjoining cells only are the chloroplasts represented. ×350.

Fig. 2. Surface view of last. ×350.

Fig. 3. Surface view of pitcher-rim, showing three surface gland-cells, surrounded by epidermal cells with downward-directed processes. ×350.

Fig. 4. Vertical section of last. ×350.

Fig. 5. Semi-diagrammatic view of the inner pitcher-wall of *Sarracenia variolaris*; *a*, attractive surface; *b*, conducting surface; *d*, detentive surface. *a'*, single hair-cell from attractive surface; *b'*, single hair-cell from conducting surface; *d'*, single hair-cell from detentive surface.

Fig. 6. Semi-diagrammatic view of the inner pitcher-wall of *Sarracenia purpurea*; *c*, glandular surface; other letters as in last.

Fig. 7. Semi-diagrammatic view of the inner pitcher-wall of *Heliamphora nutans*; *a*, attractive surface; *b*, upper conducting surface; *c*, lower conducting surface; *d*, detentive surface.

Fig. 8. Two honey-glands from attractive surface, surrounded by thick-walled neighbour-cells. ×350.

Fig. 9. Glands and hair-cells from middle of area *b* of Fig. 7. ×350.

Fig. 10. Gland from external surface of sepal. ×350.

Fig. 11. Transverse section of outer ovarian wall of *Sarracenia purpurea*, showing nectariferous papillae. ×75.

Fig. 12. Transverse section of alluring stem-gland of *Nepenthes Phyllamphora*; *a*, columnar gland-cell layer with two subjacent layers of polygonal gland-cells; *b*, basement-membrane of gland, formed of two cell-layers; *c*, vascular bundle giving off a diverticulum *d*, which ends in the base of the gland; *e*, cut ends of spiral cells. ×350.

Plate XX.

Fig. 13. Vertical section of alluring leaf-gland of *Nepenthes hybrida*; *d*, terminal bundle-cells connecting with cells of the subglandular membrane; other letters as in last. ×350.

Fig. 14. Surface view of alluring gland from tendril of *Nepenthes bicalcarata*. The epidermal cells above the gland-cavity are thick-walled, and show sudden transition to the ordinary epidermal cells. ×150.

Fig. 15. Transverse section of pedicel of *Nepenthes bicalcarata* with triradiate honey-gland. ×350.

Fig. 16. Vertical section of attractive perithecioid honey-gland from inner lid-surface of *Nepenthes Lowii*; letters as before.

Fig. 17. Surface view of attractive perithecioid honey-gland from inner lid-surface of *Nepenthes Pervillei*. ×75.

Fig. 18. Surface view of honey-gland from pedicel of *Nepenthes Pervillei*. ×75.

Fig. 19. Transverse section of one-half of sepal from *Nepenthes bicalcarata*, with honey-glands (*g*) embedded in depressions of the upper epidermis. ×75.

Fig. 20. Surface view of last. ×350.

Fig. 21. Surface view of margin of corrugated rim from *Nepenthes Hookeri* after maceration in potash solution and removal of upper epidermis and mesophyll to the line *c*. One gland-bundle *a*, has been left; *b*, exposed surface of gland; *d*, orifice of gland. ×75.

Fig. 22. Vertical section of corrugated rim from *Nepenthes khasyana*: *a*, gland-bundle; *b*, sausage-shaped gland deeply sunk in an involution of the margin, of which *d* is the orifice; *e*, oblique stomata ('transverse excrescences' of authors) on conducting surface. ×75.

Fig. 23. Transverse section of corrugated rim of *N. khasyana*: *a*, corrugations; *b*, bundles traversing these; *c*, periglandular bundles; *d*, gland-tissue; *e*, lumen formed by separation of central gland-cells. ×150.

Fig. 24. Section of a marginal tooth from *Nepenthes echinostoma* exposing marginal gland at the apex. ×5.

Figs. 25–28. Sections of corrugated margin to show comparative size of glands in species. Fig. 25, *N. Rafflesiana*; Fig. 26, *N. zeylanica*; Fig. 27, *N. Phyllamphora*; Fig. 28, *N. villosa*. ×25. (P. W. Nicol delt.)

Plate XXI.

Fig. 29. Surface view of corrugated rim from seedling-leaf of *Nepenthes edinensis*. ×75.

Fig. 30. Vertical section of corrugated margin from young pitcher of *Nepenthes hybrida*, showing rudiment of marginal gland developing in a depression of the epidermis. ×350.

Fig. 31. Vertical section of pitcher-wall of *Nepenthes khasyana*: *a*, alluring gland; *b*, one of the external vascular bundles from which a diverticulum for gland-supply has been given off; *c*, 'peptic' gland; *d*, one of the internal vascular bundles from which diverticula pass to the 'peptic' glands. ×150.

Fig. 32. Vertical section of inner pitcher-wall of *Nepenthes Veitchii*, showing 'peptic' glands lying in depressions formed by flap-like outgrowths of tissue lying above each. ×75.

Fig. 33. Surface view of detentive region from *Nepenthes sanguinea*, with three 'peptic' glands slightly covered by flaps and supplied by vascular tissue left after removal of mesophyll tissue by maceration. ×75.

Fig. 34. Surface view of detentive region from *Nepenthes Pervillei*, with 'peptic' glands deeply sunk in funnel-shaped pockets. ×75.

Fig. 35. Surface view of detentive region from *Nepenthes Lowii*. ×75.

Fig. 36. Vertical section of 'peptic' gland from *Nepenthes bicalcarata* : *a*, down-growing epidermal flap; *b*, surface columnar cell-layer of the gland; *c*, sub-glandular membrane; *d*, terminal elements of the vascular bundle. ×350.

Fig. 37. Receptacular processes from flower of *Cephalotus follicularis*. ×75.

Figs. 38–43. Leaflets of *Gleditschia triacanthos*.

Fig. 44. Teratological pitcher of *Sarracenia purpurea*.

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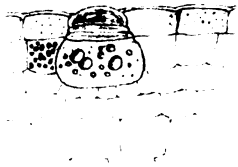

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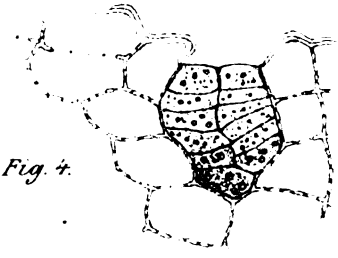

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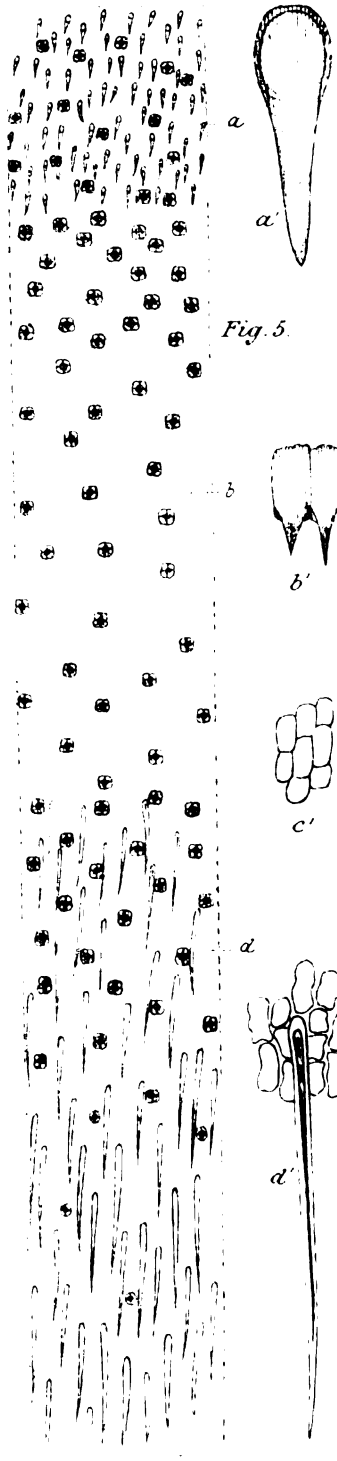

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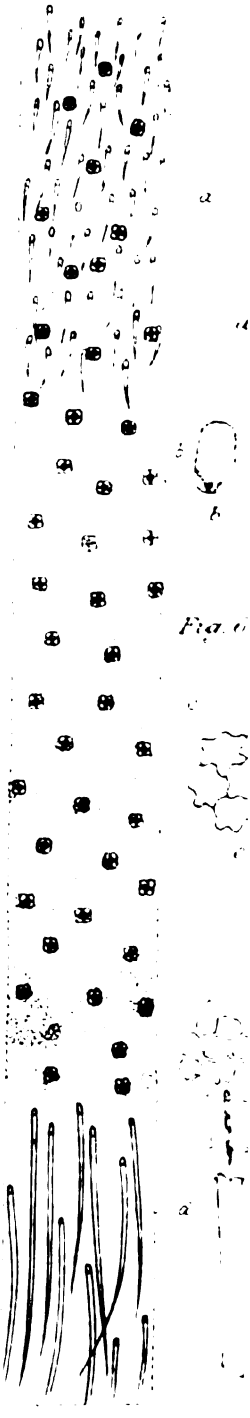

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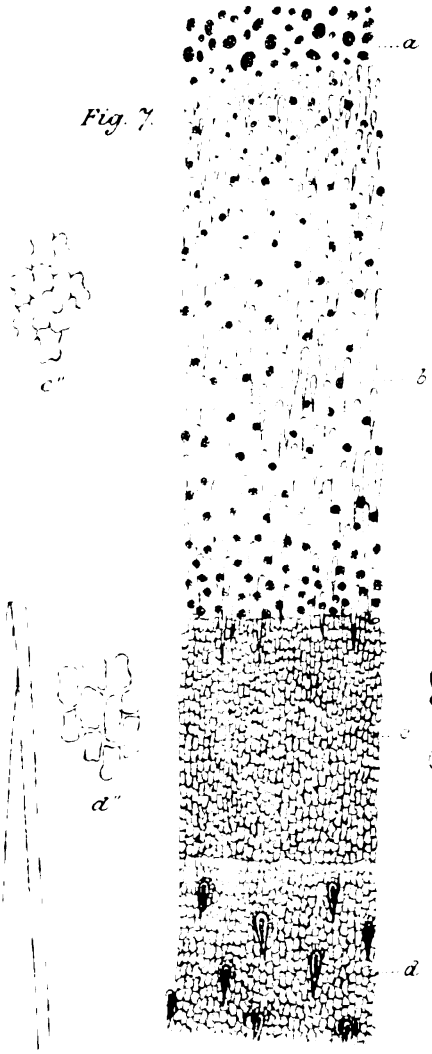

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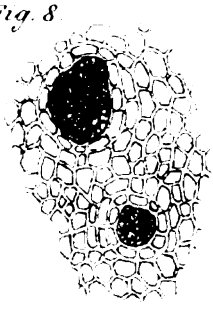

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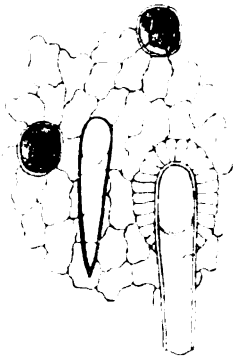

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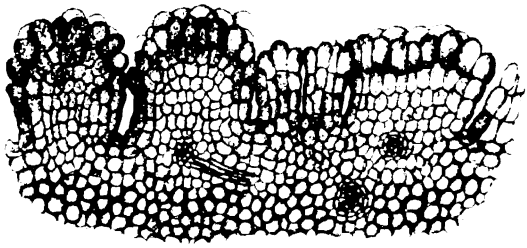

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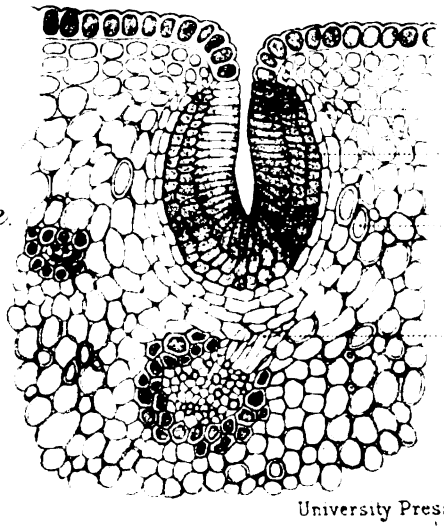

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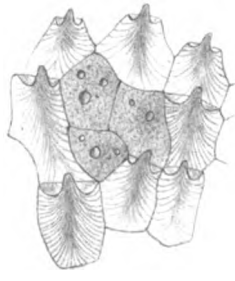

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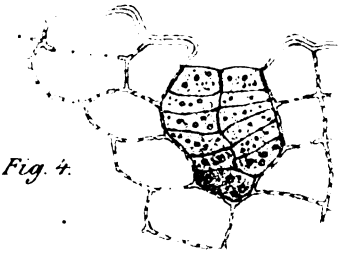

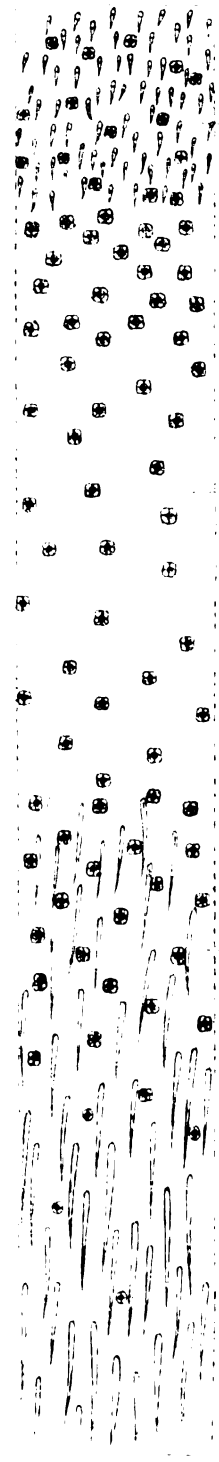

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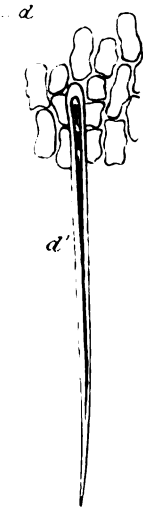

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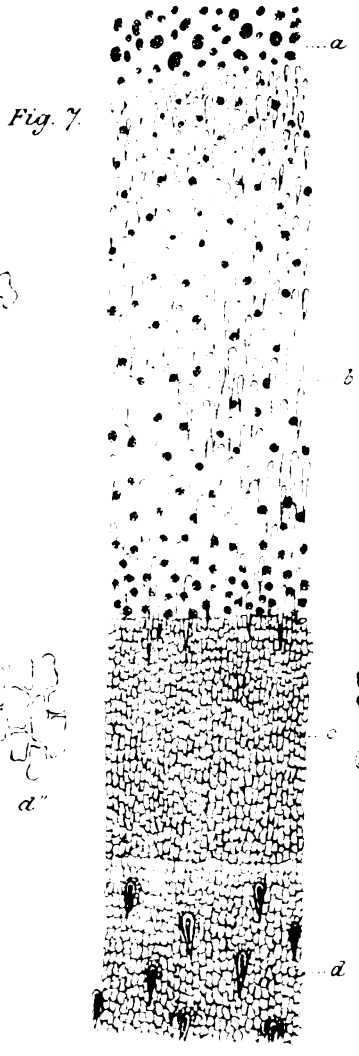

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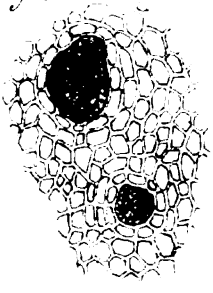

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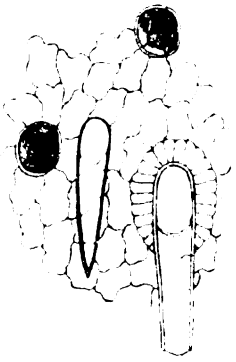

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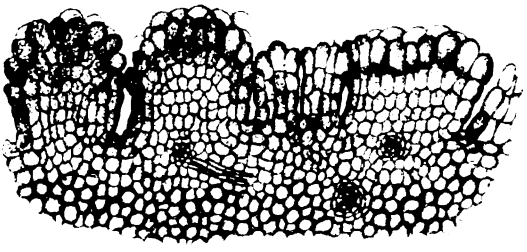

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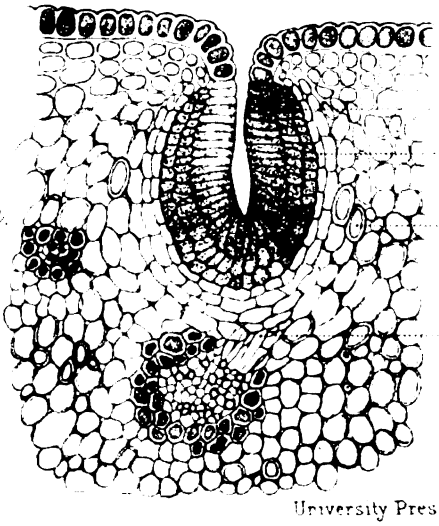

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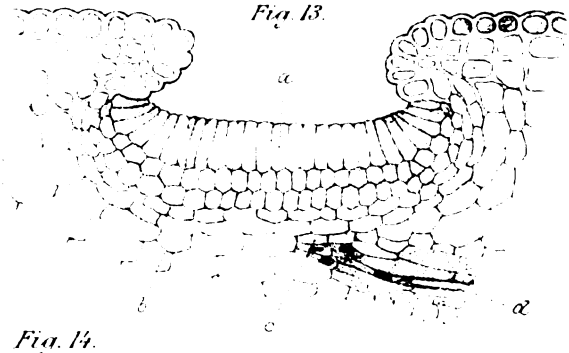

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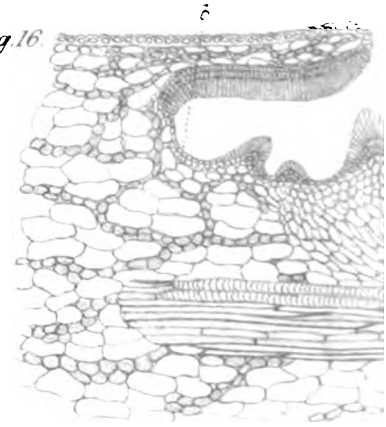

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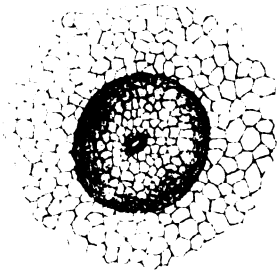

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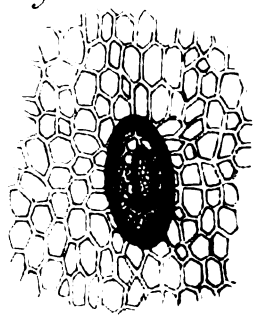

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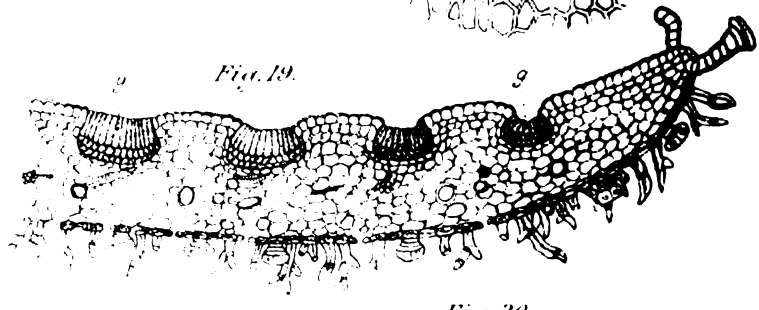

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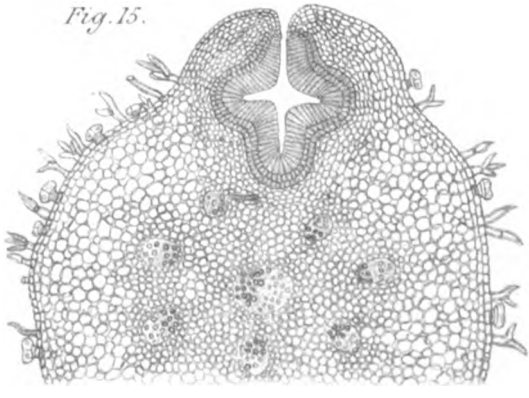

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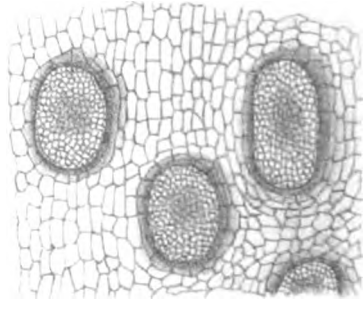

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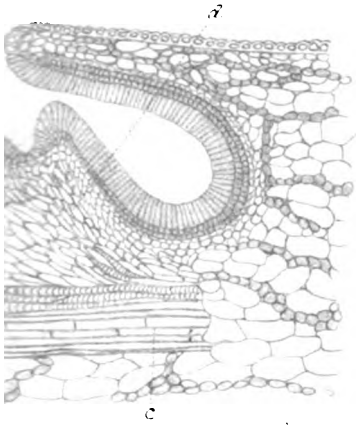

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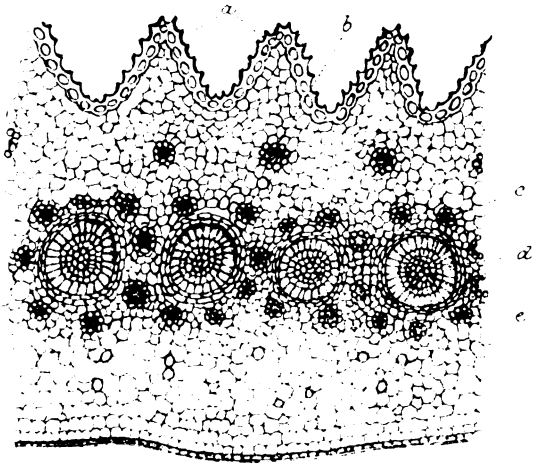

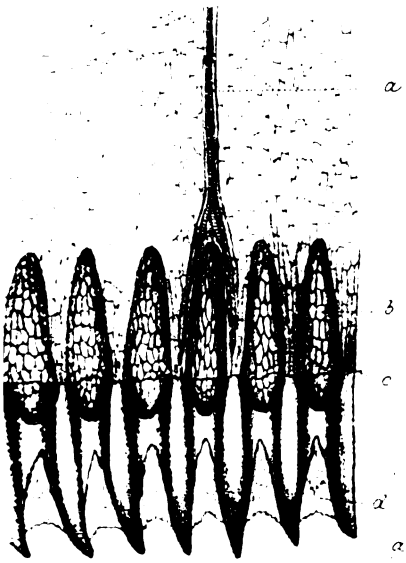

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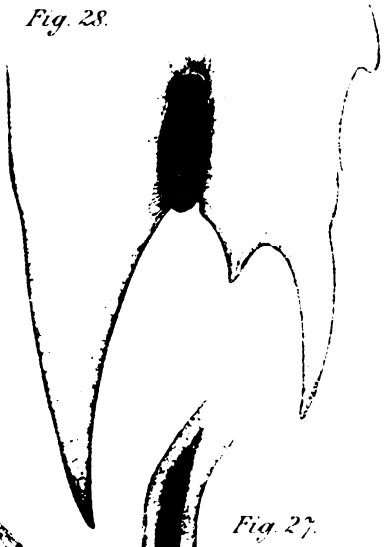

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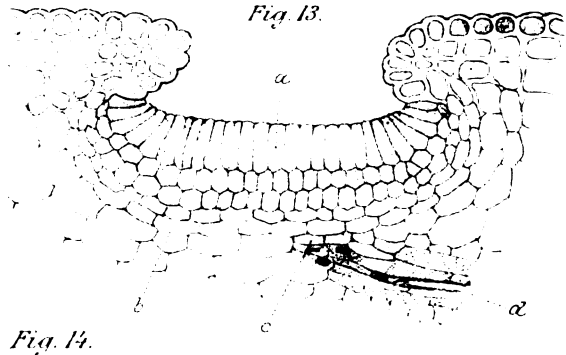

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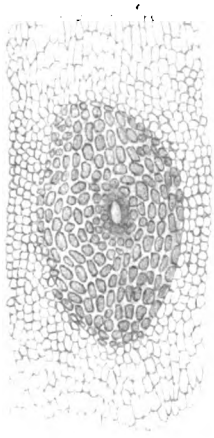

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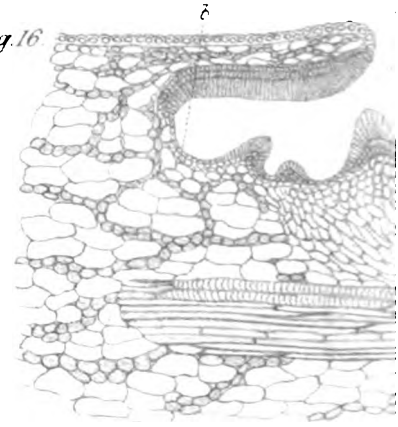

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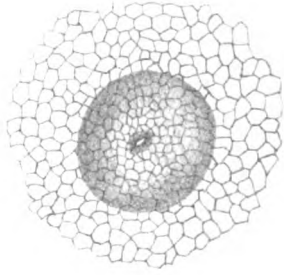

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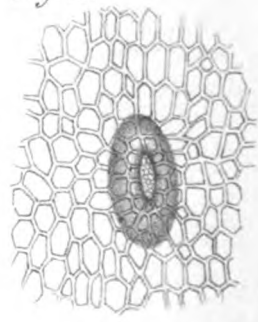

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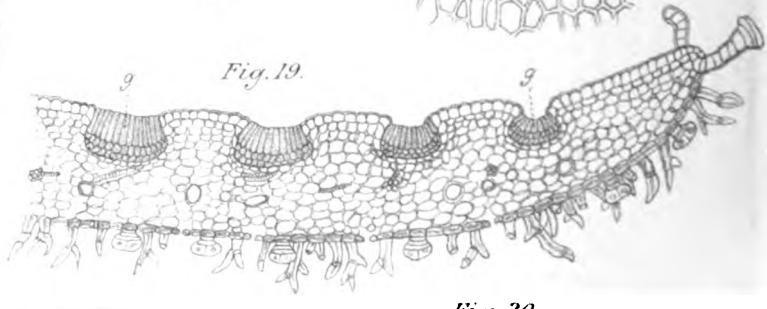

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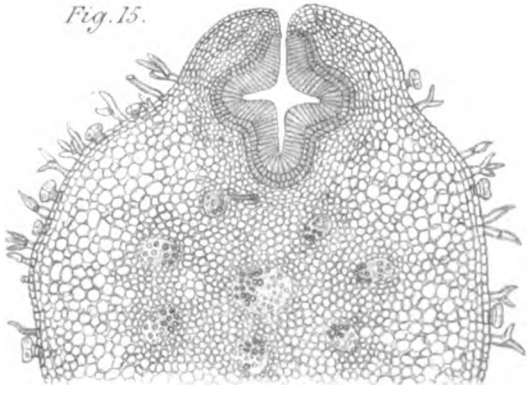

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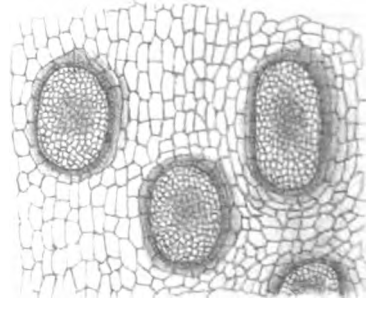

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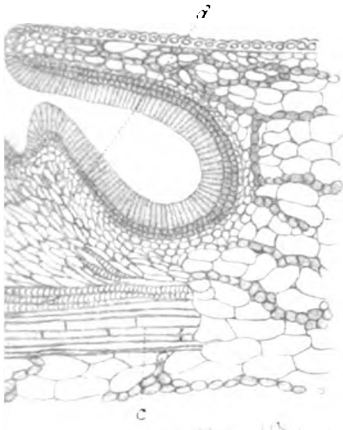

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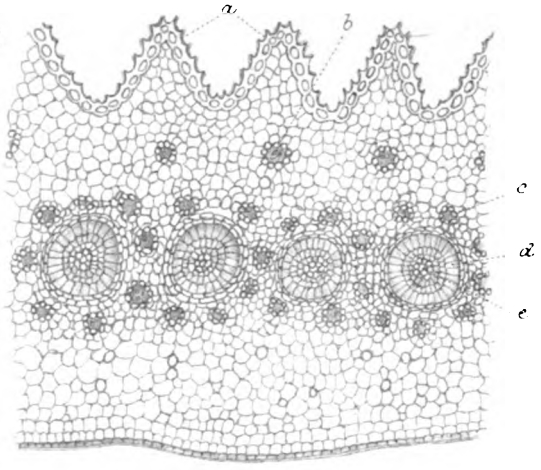

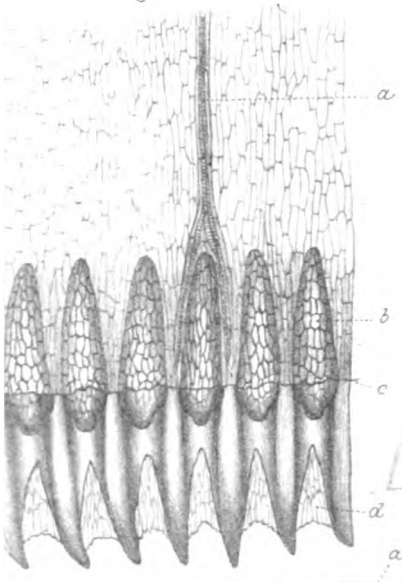

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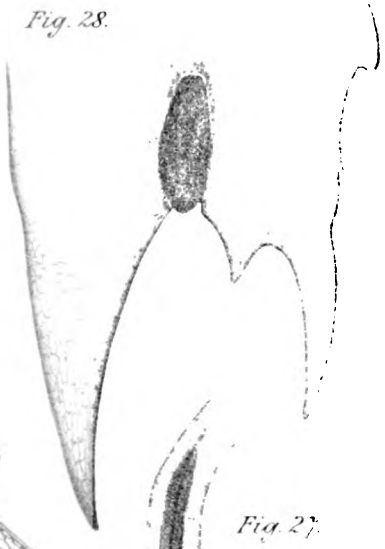

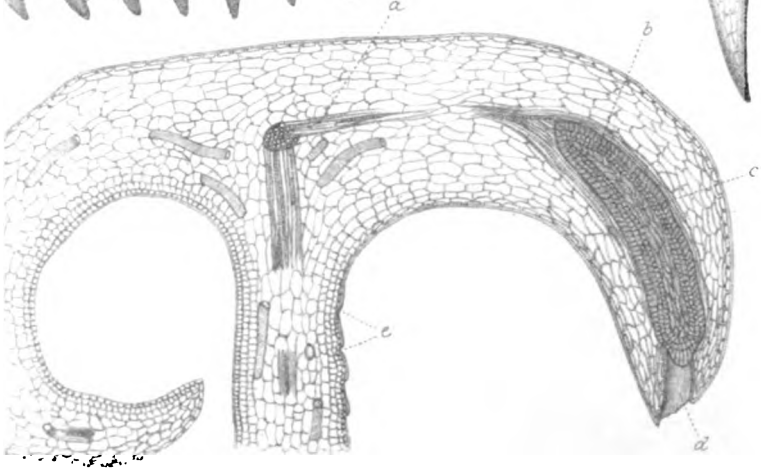

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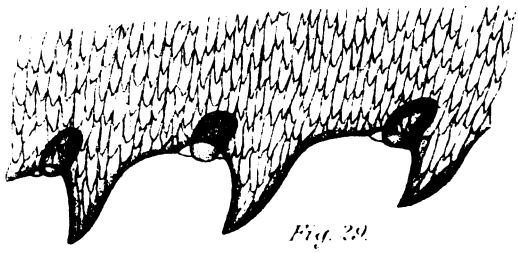

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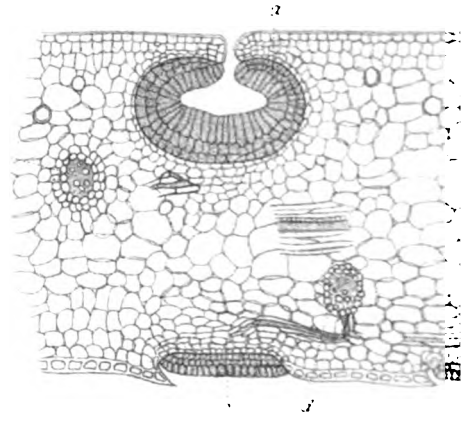

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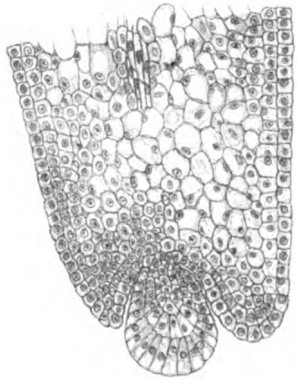

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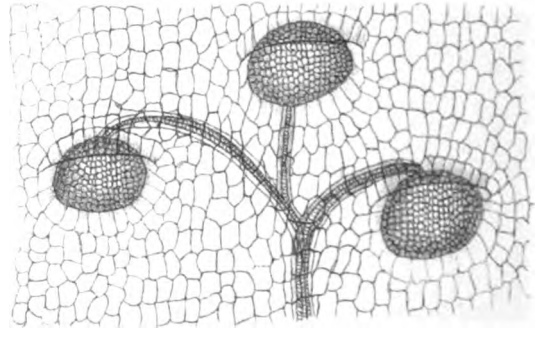

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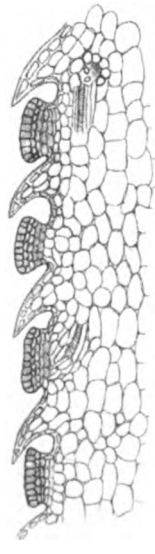

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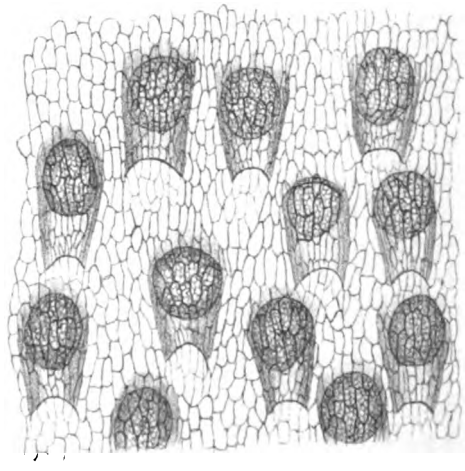

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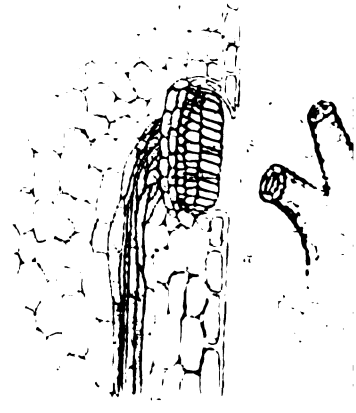

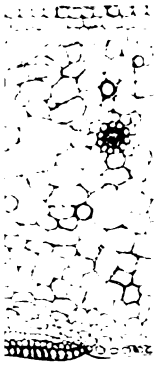

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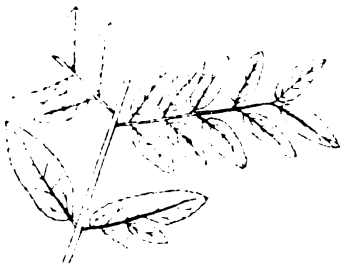

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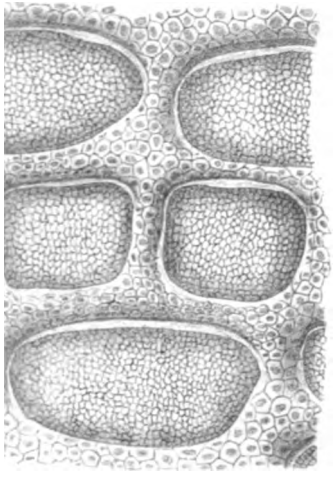

Fig. 44.

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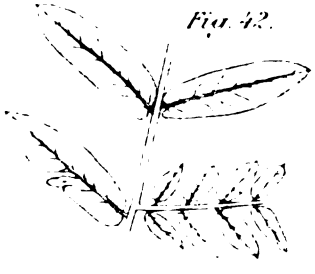

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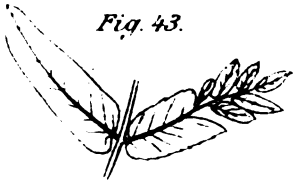

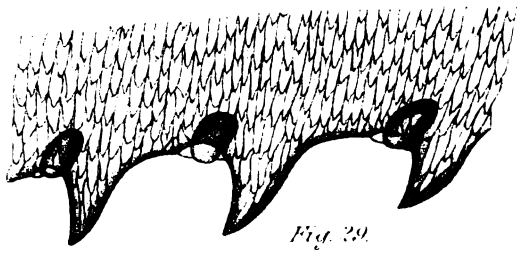

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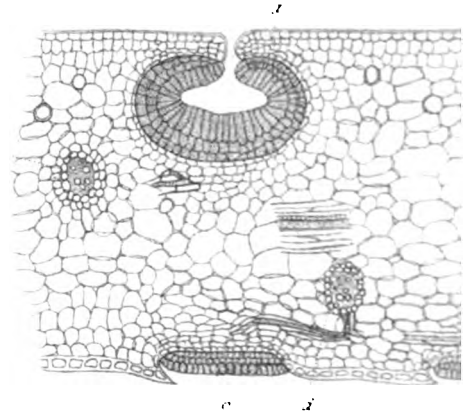

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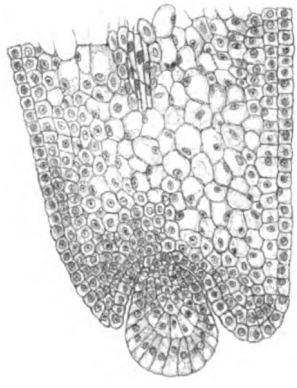

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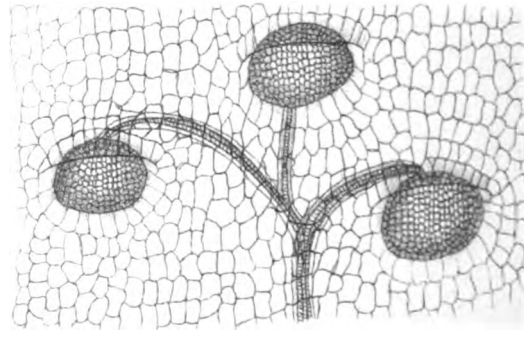

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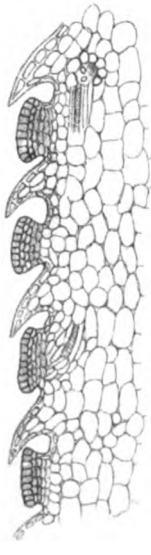

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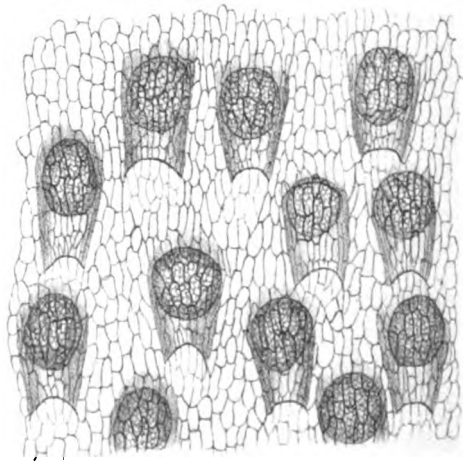

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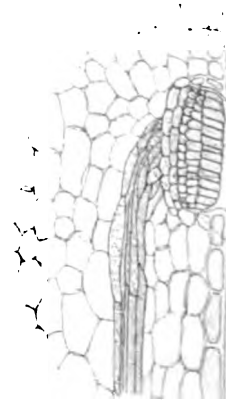

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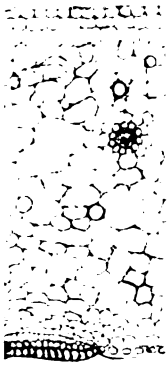

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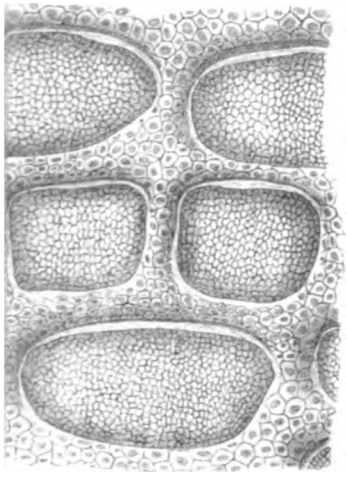

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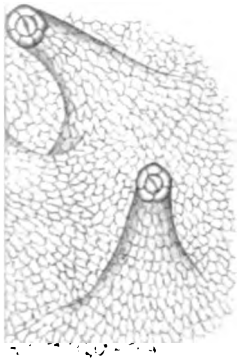

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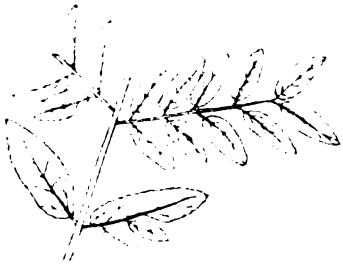

Fig. 39.

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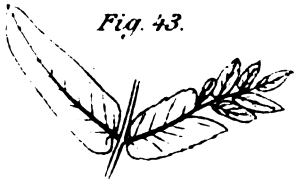

Fig. 44.

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On the Growth of the Fruit of Cucurbita.

BY

FRANCIS DARWIN.

With Plates XXII and XXIII.

THE work of which I here give an account began with an attempt to estimate the increase in weight[1] of the fruit of the Vegetable Marrow (*Cucurbita*) and of other gourds. I became interested in the subject, and passed on to the measurement of the growing fruit. This latter part of the subject is not new; Kraus[2] has made observations on Gourds, Apples, Pears, &c. But as my experiments are in some respects more detailed than those of Kraus, they seem to me worth publishing. My first attempt at estimating the increase in weight of the fruit of *Cucurbita* was an extremely rough one. I was staying in a country house, away from all means of accurate observation, but it seemed worth while to get some rough ideas of the facts. Having selected a vegetable marrow growing out of doors, and apparently increasing vigorously, I proceeded to weigh it at stated intervals with the only instrument at my disposal, namely, a spring-balance graduated to ½ oz.

[1] The desirability of investigating the rate of growth in weight was suggested to me by my friend Dr. Sherrington, of St. Thomas' Hospital.

[2] G. Kraus, Abhandl. d. Naturf. Gesellsch. zu Halle, Vol. xv.

[Annals of Botany, Vol. VII. No. XXVIII. December, 1893.]

It is not necessary to give the details; it will be enough to say that on Sept. 18, 1890, the balance recorded 20 oz.; on Sept. 22, 33¾; on Sept. 27, 62 oz. Therefore in the first four days it increased in weight at the rate of 3·44 oz. per day; in the last five days at the rate of 5·65 oz. per day. This latter rate is equivalent to about 6·8 grams per hour; it was therefore evident that, with a better balance, it would be easy to record the changes in weight at short intervals. In the autumn of 1892 I made some observations on a gourd growing in a small greenhouse. The branch of about half a meter in length on which the fruit was borne, necessarily interfered with the freedom of the balance on which the fruit was placed, but this source of error is unavoidable, and one of which it is not easy to estimate the amount or the constancy. The instrument was a French druggist's balance, capable of carrying 8 kilo, and turning with 0·5 gram when loaded with 1,000 grams on each pan.

In the following table I have expressed the rate of increase in milligrams per minute, so that the growth may be easily

EXPERIMENT I.

Time.	Grams.	Milligrams per minute.
Sept. 6, 1892.		
5.42 p.m.	704	
6.22 ,,	712	200
6.52 ,,	715	
9.11 ,,	729	100·5
Sept. 7.		
10 a.m. Few small leaves removed	776	61·1
10.6 a.m.	769	
12.53 p.m.	787	107·8
3 42 ,,	802	88·7
5.56 ,,	820	134·3
Sept. 8.		
10.0 a.m.	882	64·3
2.30 p.m.	890	29·6
5.30 ,,	897	38·9
9.21 ,,	917	86·6
Sept. 9.		
10.10 a.m.	964	61·1

comparable with the later results, for which this manner of calculating the increase proved to be convenient.

The figure which occurs in the third column opposite the first morning reading on Sept. 7, 8, 9 gives the rate of growth since the last reading on the preceding evening.

The results are of small value, but as far as they go they agree with later experiments in showing a decrease in rate in the afternoon and an increase towards evening. Also in the low average growth of the whole night.

For the following observations on the change in weight of the fruit I used a different balance, originally designed by my brother Horace as a self-recording instrument.

It consists of a beam turning on a knife-edge, at one end of which the gourd is hung in place of the scale-pan; at the other end of the beam is a scale-pan containing weights not quite sufficient to balance the fruit: the equipoise is effected by a spiral spring which supports the heavier end of the beam. If the fruit gains weight, the fruit-end of the beam will sink, and will stretch the spring until a new position of equilibrium is attained; in this way the movement of the beam represents changes in weight of the fruit. The movement of the beam is magnified by prolonging one of its arms to a length of 62 cm. from the knife-edge; this long arm ends in a fine index, and its position is read on a scale divided into millimeters.

The index is brought back to the zero by adding weight to the scale-pan. The following figures give the weight so

	Grams.	Mm.	Value of one gram.
Sept. 10, 1892.	40	111	2·8 mm.
,, 11 ,,	45	129·5	2·9 ,,
,, 12 ,,	32	89	2·8 ,,
,, 13 ,,	20	62	3·1 ,,
,, 14 ,,	40	119	3·0 ,,
,, 16 ,,	40	117	2·9 ,,
,, 18 ,,	40	103	2·6 ,,
,, 19 ,,	30	65	2·3 ? ,,
,, 20 ,,	50	147·5	3·0 ,,

added on a series of days; also the number of millimeters through which the index moved, and the calculated value in millimeters of one gram.

In calculating my results I have taken 1 gram as equal to 3 mm. on the scale.

The instrument when used for other purposes, for instance for transpiration, is fairly accurate, but when, as in the present instance, it is hampered by the springlike, and probably varying, effect of the branch on which the fruit is borne, it is but a rough apparatus. I believe, however, looking at my results as a whole, that the errors are not sufficient to cast serious doubt on the general conclusions deducible.

We have seen in Exp. 1 that the fruit which I describe in my notes as V2 was continuously increasing in weight from Sept. 6 to Sept. 9. The following table shows the remarkable fact that a loss of weight may occur. Later observations show that this is common, and that it is accompanied by a diminution in volume[1]. The change in weight is given in milligrams per minute; when the figures in the third column

EXPERIMENT II.—Sept. 10, 1892. V2.

Time.	Reading.	Rate.
	mm.	Mg. per minute.
11.6 a.m.	89	...
12.2 ,,	91	+12
12.55 ,,	98	+44
2.1 ,,	117	+95
2.33 ,,	110	−73
3.15 ,,	114	+31
4.15 ,,	119	+30
5.20 ,,	128	+46
6 ,,	136	+66
7 ,,	148	+66
8 ,,	163	+83
9 ,,	173	+55
10 ,,	181	+44
11 p.m.	188	+33

are preceded by + there is a gain in weight, while a minus sign means loss in weight.

[1] Kraus, loc. cit., has described the shrinking of various fruits.

The loss of weight occurs between 2·1 and 2·33. The chief increase in rate is from 5.20 to 8, after which a distinct fall in rate takes place. This fall is of frequent occurrence, both as to changes in weight and changes in size.

EXPERIMENT III.—Sept. 11, 1892. V2.

Time.	Reading.	Mgr. per min.	Temp. C°.
	mm.		
10.0 a.m.	155		18·3
10.30 „	155·5	+6	20·8
11.0 „	150	−60	22·5
11.30 „	149	−11	20·5
12.0 „	152	+33	19·8
12.30 p.m.	151·5	−6	19·5
1.0 „	153·5	+22	20·0
1.30 „	155	+17	20·0
2.0 „	156·5	+17	23·0
2.30 „	156	−6	22·5
3.0 „	156	0	21·5
3.30 „	157	+11	21·5
4.0 „	158	+11	21·5
4.30 „	159	+11	20·5
5.0 „	160·5	+17	20·0
5.30 „	163	+28	19·0
6.0 „	166	+33	18·0
6.30 „	169·5	+39	17·0
7.0 „	173	+39	16·5
7.40 „	180	+58	16·5
8.0 „	183·5	+58	16·5
8.30 „	189·5	+67	16·0
10.0 „	61	New zero.	15·5
10.30 „	64	+33	15·0
11.32 „	70	+32	14·5

Exp. 3 is graphically represented in Fig. 1, Plate XXII: the unbroken line represents change in weight, the broken line change in temperature. It will be seen that at 11 a.m. and 2 p.m. the temperature rises suddenly and falls again, and at both these points the growth curve falls and rises, i.e. varies in the opposite direction to the temperature curve. This is a very general occurrence, both in regard to the curve of weight and of size. It is certain, from later observation with the dry and wet-bulb thermometer, that the hygrometric state of the air is the chief factor in determining changes in the rate of growth. Therefore the contrast between the growth and

temperature curves in Fig. 1 must depend on the fact that in a greenhouse the air is, roughly speaking, drier when the temperature rises and damper when it falls. Fig. 1 also shows the afternoon rise and the nocturnal fall in growth.

EXPERIMENT IV.—Sept. 12, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	Mgr. per min.		
6.0 a.m.	117		14·5	
·30 „	111·5	−60	14·8	
7.0 „	113	+17	15·5	
·30 „	114·5	+17	16·5	
8.0 „	115	+6	19	
·30 „	112·5	−28	20	
9.0 „	109	−39	21·5	
·30 „	111·5	+28	20·5	
·50 „	114	+41	20	No sun.
10.20 „	117	+33		
·50 „	122	+56	19·5	Half sun. Blind down.
11.20 „	123·5	+17	23·5	Cloudy.
·50 „	128	+50	22·5	Ditto, blind up.
12.32 p.m.	130	+16	23·5	
1.42 „	133	+14	24·2	Sun.
2.13 „	133·5	+53	24·3	Sun.
·55 „	135	+12	22	Sun.
3.35 „	138	+25	22·5	
4.13 „	142	+35		
Altered weights.				
4.25 „	118		21·5	
5.25 „	124	+33	21	
6.25 „	133·5	+53	19	
7.25 „	147	+75	17·5	
8.25 „	162	+83	17	
9.25 „	174·5	+70	16·5	
Altered weights.				
9.37 „	85·5			
10.0 „	87	+22		
10.37 „	91·5	+41	16·5	

Fig. 2 represents Exp. 4. The same general character is shown; namely loss of weight or diminution of rate, as the case may be, occurring with rises of temperature. The evening rise is as usual followed by a decrease in rate of growth.

Exp. 5 (represented in Fig. 3) is worth giving on account of the long continued loss of weight; the fruit does not begin

to gain weight until 3.25 p.m. The variations in the rate of loss do not correspond to any striking temperature changes.

EXPERIMENT V.—Sept. 13, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	Mgr. per min.		
8.45 a.m.	68		22	
9.0 „	65			Blinds down.
9.15 „	62	−67	22·5	
9.30 „	61			
9.45 „	60	−22	21	
10.0 „	58		21·5	
10.20 „	57	−28	21·3	Cloudy.
Tested balance.				
12.13 p.m.	120			Sun.
.18 „	118		24	
Plant shaken and disturbed				
12.32 „	114·5	−83		
1.32 „	109	−30	26	Sun and cloud.
2.32 „	108	−6	26	Sun and cloud.
3.25 „	95·5	−78	24	Sun.
4.25 „	93·5	−11	24	Sun.
5.25 „	95·5	+11	20·5	Sun too low for much effect.
6.25 „	103	+42	18·5	
7.25 „	112	+50	16·5	
8.25 „	123	+62	15	
9.25 „	130	+39	15	
10.25 „	139·5	+53	14	

The rise in the curve at 1.32 corresponds with the appearance of clouds, the fall at 3.25 with unclouded sun: but these changes in the weather are not apparent in the temperature curve. It should be noted that between 10.20 and 12 the plant was much disturbed in testing the balance. This sort of disturbance generally causes a fall in the curve—why, I cannot say.

EXPERIMENT VI.—Sept. 14, 1892. V2.

Time.	Reading.	Rate.	Temp.	
	mm.	Mgr. per min.		
11.10 a.m.	336		22·5	Cloudy.
.30 „	336			
12.2 p.m.	335	+6	21·2	
.32 „	336·5		21·1	
.48 „	336·5	−11		
1.48 „	336	+3	24	
2.48 „	335·5	+3	23	Half-sun.
4.0 „	335·5	0	22	
5.0 „	335·5	0	21	Sunny, but too oblique to reach plant.
6.0 „	330·5	+28	15·5	
7.8 „	320	+50	12·5	
8.0 „	312·5	+48	11·5	
9.0 „	304	+47	11·5	
10.0 „	296	+44	10·5	
10.38 „	292·5	+30	10	

Exp. 6 shows once more the evening rise and nocturnal fall in the weight curve.

EXPERIMENT VII.—Sept. 15, 1892. V2.

Time.	Reading.	Rate.	Temp.	
8.0 a.m. Weights altered.		Mgr. per min.		
	mm			
8.10 „	364		14	Shading removed at 8 a.m., and sun shining on plant until 10.30, when shading replaced.
9.0 „	386	−146	18·5	
10.0 „	394·5	−47	21	
11.0 „	405·5	−61	20·3	
12.6 p.m.	406	−3	20·5	
12.38 „	404·5	+15	20·5	
1.0 „	406	−23	21·5	
2.0 „	406	0	20	
3.0 „	405	+6	20	Sun and cloud.
3.38 „	403	+18	19·5	Power of sun much less.
4.0 „	401	+30	19	Shading removed.
4.36 „	401	0	18·3	
5.0 „	398	+42	18·5	
5.30 „	394	+44	16	
6.0 „	388	+67	15	
6.18 „	384	+74	14·3	Twilight.
6.36 „	379·5	+83	13	
7.0 „	374	+76	12·5	
8.0 „	358·5	+86	12	
9.0 „	348	+58	11·5	
10.0 „	337·5	+58	11·5	
10.30 „	331·5	+67	11·5	

In Exp. 7 there is rapid loss of weight in the morning, diminishing in amount when the blinds are pulled down, changing to gain in weight when clouds appear at 3 p.m. At about 4 p.m. the blinds were pulled up prematurely, and growth fell to zero. As evening came on the gain in weight is rapid, but falls again at 9 p.m.

EXPERIMENT VIII.—Sept. 16, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	Mgr. per min.		
8.10 a.m.	240·5		14·0	No sun.
·20 „	239·5		15·0	
·40 „	236·5	+44	16·5	
9.0 „	234·5	+33	17·5	
·45 „	228	+48	19·3	Cloudy still.
Disturbed plant.				
10.15 „	229·5		21·0	Sun.
·40 „	235	−73	21·3	
11.0 „	241·5	−109	21·3	Sun.
·55 „	251·5	−60	23·3	Sun and cloud.
12.30 p.m.	253·5	−19	21·0	
12.59 „	257	−46	20·0	Cloud.
1.29 „	254·5	+28	18·5	Some rain.
2.1 „	250·5	+44	18·5	Cloud.
·41 „	248	+21	20·8	Faint sun.
3.42 „	249	−5	22·5	Sun.
4.13 „	249		21·0	Sun oblique.
·46 „	248		20·0	
5.19 „	246		18·0	Sun behind buildings.
·33 „	244·5	+19	17	
Added weight.				
6.0 „	365		16·0	
6.30 „	364		14·0	
7.10 „	361	+19	12·5	
8.0 „	357	+27	11·0	
9.0 „	351	+33	10·0	
10.0 „	343·5	+42	10·0	

Exp. 8 is given in Fig. 4. The opposition between the weight curve and that of temperature is very distinct. The nocturnal fall is absent.

In Exp. 9 we have loss of weight appearing when the shading was prematurely removed at about 4 p.m. The afternoon rise is rapid and is followed as usual by a fall.

The vegetable marrow on which the above observations were made was finally placed on the druggist's balance on Sept. 22, when it weighed 1460 grams; on Sept. 6 it weighed

EXPERIMENT IX.—Sept. 17, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	Mgr. per min.		
8.6 a.m.	286		12·5	Sun: shading taken off.
8.15 „	287		15·5	
8.45 „	291·5	−47	19·5	Bright sun.
9.45 „	307·5	−89	25	Shading replaced.
10.15 „	311	−39	19	Shading increased,
10.21 „	314			Sun. [door opened.
10.59 „	319	−61	18·3	
11.46 „	322·5	−25	19·5	Sun.
12.0 „	322·5			
12.35 p.m.	326	−24	19·5	Sun.
1.17 „	328	−16	20	
2.0 „	329		19·5	Bright sun.
2.30 „	330	−9	19	
3.30 „	331	−6	18	Bright sun.
4.0 „	331	0	17	Sun nearly off: shading removed.
4.15 „	332	−22	18	
5.0 „	329	+22	15·5	
5.10 „	326		14	Sun off.
5.25 „	320		13·5	
5.35 „	319	+95	13·5	
6.5 „	304·5	+161	12	
7.5 „	283·5	+117	9·5	
8.10 „	262	+110	9·5	
9.30 „	244	+75	8·5	

704 grams, so that in 16 days it gained 756 grams, or about 33 mg. per minute.

Increase and Decrease in the size of Cucurbita Fruits.—The observations were made with the apparatus designed by Mr. H. Darwin and used by Miss Anna Bateson in her paper on the change of shape in turgescent pith[1]. A vertical micrometer screw graduated to 0·01 mm. carries at its lower end a vertical needle. The micrometer is supported on a strong retort-stand, and is so arranged that the needle-point is over the centre of the gourd, which rests on a firm support. A minute ebonite vessel, 5 or 6 mm. in

[1] Annals of Botany, Vol. IV.

diameter, is filled with oil and placed on the surface of the gourd, vertically beneath the needle-point. The micrometer is now screwed down until the point comes in contact with the oil, a moment which is very definitely seen. Any change in the vertical diameter of the fruit is of course perceptible in the micrometer readings. With practice the micrometer is reliable to 0·01 mm., and a third decimal place can be estimated. For some reasons it would be better to use mercury for the reflecting surface, but contact with oil is far more easily seen, and the error arising from evaporation or clogging of oil is spread over many days.

Other sources of error are more serious. The fruit-bearing branch must be securely tied in several places to heavy retort-stands, or to stakes stuck in the soil; if this is not done the fruit will not be steady. I can only say that I was fully alive to this possible source of error from the first. The fruit rested on a glass plate supported on an iron tripod; the ebonite pot was steadied on the curved surface of the fruit by a bedding of putty. As the fruit grew, the oil-pot gradually moved laterally with regard to the needle, so that at intervals of about twenty-four hours it was necessary to recentre it.

Another source of error is the expansion by heat of the iron rod which supports the micrometer. When the rod expands the readings of the micrometer will show an apparent shrinking of the fruit, and *vice versa*.

I have not given the correction for temperature; the following examples show the extent of error.

July 12, 1893. The vertical height of the fruit (which gives the length of iron rod whose expansion has to be allowed for) was 113 mm. In round numbers the expansion of iron for 1°C. is 0·00001 of a unit of length.

The following readings give a fair range of temperature.

	Microm.	Temp.
5 p.m.	4·615	24·6
11 „	7·430	17·5

The rate of growth without correction for temperature is 7·82 μ per minute, the corrected rate is 7·79. As I only give

one place of decimals, these would both have been entered as 7·8. In the following instances I have picked out cases where the temperature varied several degrees in a short time.

July 12.

Time.	Microm.	T.	Uncorrected rate.	Corrected rate.
11.40 a.m.	2·395	25·0		
12.48 „	2·960	22·1	8·31	8·26
	Would be entered as		8·3	8·3

July 13. Fruit 123 mm. in diameter.

12.34 a.m.	12·645	21·7		
12.46 „	12·748	19·9	8·58	8·38
Would have been entered as			8·6	8·4

Sept. 25, 1892. Diameter 140 mm., taken as 150.

9.0 a.m.	8·020	16·7		
9.30 „	7·985	18·5	1·17	1·27
Would have been entered as			1·2	1·3

Sept. 24, 1892.

10.2 a.m.	8·845	19·8		
10.34 „	8·745	23·8	3·12	3·31
3.22 p.m.	8·480	20·3		
3.43 „	8·473	19·1	0·33	0·247

On the whole it may be taken that the temperature correction makes only a small difference in the decimal place, and I have never attached importance to the decimal place.

I have expressed the rate of growth of the fruit in 0·001 mm. (μ) per minute, not from any exaggerated belief in the accuracy of my results, but merely because it is the least laborious for calculation[1].

[1] To facilitate calculation final cyphers were added to some of my original notes. It thus unfortunately happens that in my published results three places of decimals are given where only two should appear.

Exp. 10. A well-grown gourd (V1), cultivated out of doors.

EXPERIMENT X.—Sept. 29, 1891. V1.

Time.	Reading.	Rate.
	mm.	μ per min.
8.33 a.m.	13·01	+ 1·8 (night)
9.36 ,,	13·11	+ 1·6
10.20 ,,	13·16	+ 1·1
12.4 p.m.	13·19	+ 0·3
1.3 ,,	13·18	− 0·2
2.18 ,,	13·26	+ 1·1
3.30 ,,	13·325	+ 0·9
5.16 ,,	13·56	+ 2·2
6.47 ,,	13·71	+ 1·6
8.50 ,,	13·99	+ 2·3
9.48 ,,	14·12	+ 2·2

It shows the same general features as those seen in the weight curve, namely, a fall in growth and shrinking in the middle of the day, with a rapid rise in the evening.

For the following experiments the *Cucurbita* was cultivated

TABLE A.

Time.	Reading.	Rate.
Sept. 6, 1892.	mm.	·01 mm. per hr.
10.25 a.m.	5·82	
Sept. 7.		
9.48 a.m.	8·15	10·0
10.57 ,,	8·28	
Rearranged micrometer.		
11.31 a.m.	6·26	
Sept. 8.		
9.44 a.m.	8·20	8·8
Sept. 9.		
10.12 a.m.	9·98	7·3
Sept. 10.		
10.18 a.m.	11·26	5·3
Rearranged micrometer.		
Sept. 12.		
9.47 a.m.	9·55	
Sept. 13.		
10.28 a.m.	10·45	3·6
Sept. 14.		
10.15 a.m.	10·73	1·2

in a small cool greenhouse, which, though it answered well enough, was inconvenient in one important respect, namely, that it had no proper blinds, so that the shading had to be effected by mats or other material thrown on the roof. The plant was kept under observation for eight days, during which the rate of growth showed a steady decrease, as if it were the end of a grand period. (See Table A on p. 471).

I do not give the details of all these days; Exp. 11 gives a fairly typical day.

EXPERIMENT XI.—Sept. 8, 1892. V 5.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	μ per min.		
9.55 a.m.	8·21	...	21·5	Bright sun, blinds down
10.10 ,,	·243	+3·3	21·0	9.44 a.m.
.42 ,,	·32	2·4	20·3	Sunny.
.56 ,,	·35	2·1	19·5	
11.11 ,,	·37	1·3	19·8	
12.2 p.m.	·432	1·2	20·0	Occasional clouds.
.23 ,,	·445	0·6	20·0	Sun shining on leaves, the blinds altered so as to shade them.
.44 ,,	·462	0·8	20·0	
1.30 ,,	·525	1·4	19·3	Sun and cloud.
2.3 ,,	·57	1·4	20·0	Sun.
.23 ,,	?			Table shaken, reading therefore doubtful.
.43 ,,	·640		19·5	Clouded.
3.3 ,,	·66	1·0	19·5	Sunny.
.44 ,,	·70	1·0	20·5	
4.5 ,,	·722	1·0	19·9	
.34 ,,	·745	0·8	20·0	Sunny.
5.13 ,,	·820	1·9		
.23 ,,				Sun behind trees.
.36 ,,	·890	3·0	18·8	Lamp lighted to keep the house warm.
.56 ,,	·940	2·5	16·3	Roof covered with mats:
6.25 ,,	·995	1·9	16·0	fairly dark.
9.14 ,,	9·21	1·2	11·5	

Fig. 5 gives the result graphically. The dip in the curve at 12.23, when the sun shone on the leaves, should be noted; also the sudden rise when the sun was off the house; also the marked nocturnal fall.

In order to ascertain whether similar growth-changes are

perceptible in the longitudinal direction, the fruit V 5 was on Sept. 15 suspended to an iron stand so that its longitudinal axis was vertical; a vertical needle was hung to its lower end, by which readings could be taken with a cup of oil moved vertically by the micrometer screw.

EXPERIMENT XII.—Sept. 16. V_5.

Time.	Reading.	Rate.	Temp. C°.	
	mm.	μ per min.		
10.1 a.m.	8·495		20·2	Sun and cloud; mats on
.15 ,,	·522	−1·9	21·0	at 10.6.
.38 ,,	·590	−2·9	21·3	Sun.
1.0 p.m.	·690	−0·7	20	Cloud.
.40 ,,	·570	+3·0	18·5	Cloud and occasional
2.4 ,,	·538	+1·3		rain.
.43 ,,	·520	+0·5	20·8	Sun and cloud.
Plant disturbed.				
3.52 ,,	·580	New zero.	22·3	
4.16 ,,	·560	+0·8	21·0	Cloud (4 hr. 8 m.)
.32 ,,	·520	+2·5		Oblique sun.
5.0 ,,	·500	+0·7	19·5	Shade.
.24 ,,	·460	+1·6	17·8	
.45 ,,	·430	+1·4	16·5	
6.0 ,,	·410	+1·3		

It will be seen (Exp. 12) that the fruit shrunk in the morning with sunshine, and elongated at 1.40 with cloud and rain. The rate diminishes at 2.43 with sunshine, rises at 4.32 after the clouds at 4.8, and falls also after the sun at 5.0; finally rises in the evening.

The next experiments were made on the fruit V 2, which had been used for the weighing experiments. The growth had practically ceased, as shown in Table B, p. 474. The second column gives the readings of the micrometer, the third and fourth columns give gain and loss in size.

Exp. 13 being the first in which readings of the wet-bulb thermometer were taken, I have given the notes in full. In most other cases I have only given the readings (chosen as dividing the time equally) from which the published rates are calculated. The column headed 'Psychr.' gives the relative humidity of the air taken from Jelinek's tables.

TABLE B.

Sept. 22.	mm.	Increase.	Decrease.
12.12 p.m.	7·295		
10.33 ,,	7·885	·590	
Sept. 23.			
8.0 a.m.	8·070	·185	
10.26 ,,	7·890		·180
10.54 ,,	8·740	New zero.	
3.12 p.m.	8·461		·279
10.30 ,,	8·875	·414	
Sept. 24.			
8.1 a.m.	8·955	·080	
4.25 p.m.	8·435		·520
10.30 ,,	8·870	·435	
Sept. 25.			
8.0 a.m.	8·940	·070	
2.0 p.m.	8·400		·540
10.0 ,,	8·960	·560	
Sept. 26.			
8 a.m.	9·015	·055	
		2·389	1·519

Total gain 0·870 mm.

EXPERIMENT XIII.—Sept. 23, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
8.0 a.m.	8·070				
·3 ,,	·070		15·0		Dull weather. No shading.
·28 ,,	·055				
·30 ,,	·055	−0·5	15		
·59 ,,	·020				
9.0 ,,	·020	−1·2	16·7		
·23 ,,	·010				
·30 ,,	7·985	−1·2	18·5		
·31 ,,	·985				
·58 ,,	·940				
10.0 ,,	·945	−1·3	18·2		Cloudy.
·16 ,,	·920				
·19 ,,	·905				
·26 ,,	·890	−2·1	19·5		
Rearranged apparatus.					
10.54 ,,	8·740	New zero.			
·57 ,,	·733				
11 0 ,,	·730		21·0		
·5 ,,	·725				
·18 ,,	·713	−1·1	21·5	92	Cloudy.
·23 ,,	·703				
·31 ,,	·695				
·36·5 ,,	·690				
·42 ,,	·685	−1·2	21·4	90	
·44 ,,	·682				
·52 ,,	·663		19·6	85	

EXPERIMENT XIII (*continued*).

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
11.55 ,,	·657				
·58 ,,	·653				
12.2 p.m.	·645	−2·0	19·4	86	
·8 ,,	·637				
·20 ,,	·622		20·4	85	Faint sun.
·24 ,,	·615	−1·4			
·28 ,,	·600				Position of thermometer altered.
·32·5 ,,	·593		21·2	87	Sun brighter, but still clouded.
·36 ,,	·580				
·38 ,,	·575				Bright sun.
·41 ,,	·575	−2·4	22·6	85	
·43 ,,					Shading put on.
·46 ,,	·520				
·49 ,,	·515	−7·5	21·6	78	
·52 ,,	·505				
·59 ,,	·500				
1.19 ,,	·495	−0·7	21·0	79	Sun and cloud.
·44 ,,	·500	+0·2	20·5	80	Thermometer in new position.
·58 ,,	·490				
·59 ,,	·493				
2.1 ,,	·490	−0·6			
·4 ,,	·483	−2·3	21·8	80	Sun and cloud.
·34 ,,	·482	0	21·5	80	
·38 ,,	·483	0			
3.0 ,,	·462	−1·0	22·1	78	
·12 ,,	·461				
·30 ,,	·470	+0·3	22·1	77	
4.0 ,,	·495	+0·8	20·5	83	Clouded : shading removed.
·30 ,,	·540	+1·5	18·7	86	
5.0 ,,	·575	+1·2	18·2	88	
·5 ,,	·595				
·10 ,,	·605	+3·0			
·15 ,,	·610		17·9	88	
·25 ,,	·635				
·30 ,,	·640	+1·8	17·4	89	
·35 ,,	·645				
·45 ,,	·665	+1·7	17·4	89	
6.15 ,,	·710	+1·5			
·17 ,,	·725				
·19 ,,	·735		16·4	94	
·42 ,,	·745	+1·3			
·43 ,,	·755				
·46 ,,	·770				
·48 ,,	·775		15·8	94	
7.28 ,,	·820	+1·6			
·30 ,,	·825		14·8	94	
·34 ,,	·825				
8.28 ,,	·845	+0·4	13·0	97	
·31 ,,	·850				
9.30 ,,	·870	+0·4	14·0	97	
·33 ,,	·865				
·34 ,,	·870				
10.27 ,,	·872				
·30 ,,	·875	+0·1	13·9	99	

From 8 a.m. to 10.26 the rate of shrinking increases; then (11.18) diminishes for unknown reasons; 12.49, increases greatly with bright sun. A permanent swelling of the fruit begins at 3.30 with the appearance of clouds; the rate of swelling reaches a maximum at 5.10, and falls to practically zero by 10 p.m.

EXPERIMENT XIV.—Sept. 24, 1892. V2.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
7.58 a.m.	8·947		15·0	97	Raining.
8.1 ,,	·960	+4·3			
·31 ,,	·960	0	15·7	97	
9.1 ,,	·940	−0·7	17·1	97	
·30 ,,	·885	−1·9	18·1	93	
10.2 ,,	·845	−1·3	19·8	91	Cloudy.
·34 ,,	·745	−3·1	23·8	77	Sun on fruit and on thermometer, which is now moved.
·36 ,,					Shading put on, and dooropened; cloudy.
·40 ,,	·705	−6·7			
11.11 ,,	·640	−2·1	21·0	84	Faint sun.
·47 ,,	·610	−0·8	21·6	80	Sun and cloud.
12.15 p.m.	·595	−0·5	22·3	76	Cloud.
·43 ,,	·560	−1·3			Sun.
1.18 ,,	·565	+0·1	21·5	72	Sun.
·45 ,,	·560	−0·2	22·5	77	Sun.
2.28 ,,	·549	−0·3	23·2	76	
·59 ,,	·510	−1·3	22·8	69	Sun.
Plant disturbed.					
3.8 p.m.	8·495				
.22 ,,	·480	−1·1	20·3	64	Sun.
·43 ,,	·473	−0·3	19·1	65	Sun.
4.25 ,,	·435	−0·9			
·35 ,,	·450	+1·5	16·8	70	
·50 ,,	·465	+1·0			
5.0 ,,	·475	+1·0	16·0	72	Sun off house; shading removed.
.10 ,,	·485	+1·0			
.20 ,,	·520	+3·5	15·5	76	
.30 ,,	·580	+6·0	14·9	80	
·55 ,,	·625	+1·8	13·9	91	
6.5 ,,	·660	+3·5			
·30 ,,	·705	+1·8	13·2	90	
·56 ,,	·745	+1·7	12·2	94	
8.0 ,,	·810	+1·0	10·5	95	
·55 ,,	·840	+0·5	9·6	96	
9.58 ,,	·860	+0·3	9·5	96	
10.30 ,,	·870	+0·3	9·4	96	

The next series of measurements were made on a fruit (V6) 63 mm. in diameter, growing in a greenhouse running north

and south, and provided with good blinds on the east and west sides.

I give Exp. 15 in a simplified form: thermometer readings were omitted.

EXPERIMENT XV.—June 15, 1893. V 6.

Time.	Rate.
	μ per minute.
10.57 a.m.	+ 1·9
11.22 ,,	2·3
·46 ,,	3·1
12.23 p.m.	5·0
2.16 ,,	0·8
·41 ,,	1·2
·54 ,,	2·6
3.30 ,,	0·6
·46 ,,	1·2
4.34 ,,	0·4
5.5 ,,	−0·6
·38 ,,	+ 0·3
6.5 ,,	0·5
·35 ,,	2·2
7.0 ,,	5·2
·28 ,,	5·7
8.1 ,,	3·1
·27 ,,	2·6
9.5 ,,	6·3
·36 ,,	4·0

EXPERIMENT XVI.—June 16, 1893. V6.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
9.34 a.m.	10·190				Sun. East blind down.
·43 ,,	·238	5·3			
·53 ,,	·280	4·2	23·6	81	
10.0 ,,	·300	2·9			
·33 ,,	·375	2·3	25·7	76	
11.3 ,,	·420	1·5	27·3	75	
·40·5 ,,	·463	1·1			West blind down.
12.7 p.m.	·512	1·8	28·8	69	
·38 ,,	·570	1·9	27·0	69	Door open.
·43 ,,	·565	New zero.	30·2	54	
4.43 ,,	11·245	2·8			
·46 ,,	·090	New zero.			Still sunny.
5.20 ,,	·230	4·1	28·8	68	
·50 ,,	·315	2·8	28·5	60	
·20 ,,	·415	3·3	27·5	63	
·40 ,,	·490	3·8			
7.0 ,,	·575	4·3			

Exp. 16 is given in Fig. 6; it shows a general but not an exact relationship between the curves of humidity and growth.

EXPERIMENT XVII.—June 21, 1893. V 6.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
6.20 a.m.	8·57		14·4	99	Cloudy.
7.0 ,,	·68	+ 2·7			
8.0 ,,	·81	+ 2·1	17·0	97	
9.5 ,,	·77	− 0·6	18·5	83	
·40 ,,	·71	− 1·7	21·7	70	
·45 ,,	·60	New zero.			
10.15 ,,	·62	+ 0·7	24·4	71	Sun.
·45 ,,	·56	− 2·0	26·4	68	House watered; blinds down.
11.17 ,,	·71	+ 4·6	22·7	76	Cloudy.
·45 ,,	·86	+ 5·3	20·2	80	Blinds up.
12.15 p.m.	9·05	+ 6·3	19·2	81	
·46 ,,	·19	+ 4·5	17·7	87	Rain.
2.45 ,,	·25	+ 0·5	19·0	85	Cloudy.
3.15 ,,	·29	+ 1·3	18·7	84	
3.45 ,,	·27	− 0·6	20·3	83	
4.15 ,,	·27	0·0	19·7	84	
·45 ,,	·27	0·0	19·5	84	
5.30 ,,	·35	+ 1·7	18·8	88	
6.0 ,,	·43	2·6	17·9	88	
·30 ,,	·56	4·3	17·0	96	
7.0 ,,	·65	3·0	16·4	98	
·30 ,,	·72	2·3	16·0	98	
8.0 ,,	·79	2·3			
·30 ,,	·90	3·6	15·6	99	
9.0 ,,	·98	2·6	15·5	99	
10.0 ,,	10·12	2·3	15·0	100	

Exp. 17, which is given in Fig. 7, Pl. XXIII, shows a closer parallelism of the curves.

The next and final series was made on a vigorous gourd, V7[1], grown in the propagating pit in which V6 was cultivated. I have given it in some detail, because I obtained a continuous series of readings for two nights and two days.

[1] On July 12, 1893, the vertical diameter was 113 mm.

EXPERIMENT XVIII.—July 12, 13, 14, 15, 1893. V7.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
July 12.	mm.	μ per min.			
11.15 a.m.	2·240		23·6	79	No blinds.
·30 ,,	·310	4·7	23·4	81	
·40 ,,	·395	8·5	25·0	83	Gleams of sun.
·46 ,,	·440	7·5			
·48 ,,	·458	9·0			
12.23 p.m.	·720	7·5	24·9	78	Rain.
·35 ,,	·823	8·6	23·1	83	
·48 ,,	·960	10·5	22·1	86	
·53 ,,	3·013	10·6			Rain.
·59 ,,	·082	11·5			
1.0 ,,	·085				
.8 ,,	·175	10·3	21·3	88	
.20 ,,	·293	9·8	20·8	89	
·30 ,,	·381	8·8			
·35 ,,	·430	9·8			
·38 ,,	·626	New zero.			
·44 ,,	·675	8·1	20·5	90	
2.29 ,,	4·000	7·2	19·6	90	Rain.
·44 ,,	·090	6·0			
3.0·5 ,,	·192	6·1			
4.0 ,,	·425	3·9	25·4	85	Bright.
5.0 ,,	·615	3·2	24·6	77	Dull; rain 5.10 p.m.
6.0 ,,	5·105	8·2	21·4	86	Rain.
7.0 ,,	·600	8·3	20·2	90	Cloudy.
8.0 ,,	6·075	7·9	19·3	91	
9.0 ,,	·595	8·7	18·2	93	
10.0 ,,	7·036	7·3	17·4	93	
11.0 ,,	·430	6·6	17·5	91	
July 13.					
1.0 a.m.	8·210	6·5	16·5	86	
3.0 ,,	9·030	6·8	16·1	90	Getting light.
4.0 ,,	·370	5·7	15·9	91	Light: rain.
5.0 ,,	·770	6·7	15·9	90	
6.15 ,,	10·200	5·7	17·1	94	Rain.
7.10 ,,	·510	5·6	17·8	92	
8.0 ,,	·770	5·2	17·8	93	Rain.
9.0 ,,	11·170	6·7	17·9	92	Rain.
·30 ,,	·380	7·0	18·3	92	
·47 ,,	·520	8·2			Rain.
10.7 ,,	·660	7·6	19·5	91	
11.0 ,,	12·020	6·8	19·2	91	No rain: cloudy.
.8 ,,	·080	7·5			
·52 ,,	·355	6·3	23·1	88	Brighter: cloudy.
12.2 p.m.	·395	4·0			
·13·5 ,,	·478	7·2	21.6	82	
·23·5 ,,	·568	9·0	22·1	84	
·34 ,,	·645	7·4	21·7	82	
·37 ,,			21·1	82	Blinds down: some
·46 ,,	·748	8.6	19·9	87	rain.
·47 ,,					Blinds up.
·49 ,,			19·7	88	

EXPERIMENT XVIII (*continued*).

Time.	Reading.	Rate.	Temp. C°.	Psych.	
p.m.	mm.	μ per min.			
12.59·5 „	12·870	9·0	19·3	90	Slight rain.
1.50 „	13·278	8·1	18·2	91	No rain.
2.37 „	·510	4·9	20·0	88	
3.9 „	·676	5·2	19·7	90	
.12·5 „	0·298	New zero.			
·54 „	·527	5·5	19·0	90	
4.11·5 „	·624	6·6	19·2	90	
.28 „	·736	5·7			
.41·5 „	·813	5·7	19·8	89	
5.10 „	·945	4·5	19·1	91	Clouds: no rain.
6.0 „	1·163	4·4	18·9	90	
7.0 „	1·445	4·7	18·4	90	
8.0 „	·803	6·0	17·0	93	
9.0 „	2·195	6·5	16·4	95	Nearly dark.
10.0 „	·475	4·7	16·2	94	
·3 „	·480	New zero.			
·57 „	·690	3·9	15·8	93	
11.8 „	·730	3·6			
July 14.					
12.20 a.m.	3·045	4·4	15·1	96	
·32 „	·086	3·4			
1·33 „	·290	3·3	15·2	96	
·43 „	·330	4·0			
2.28 „	·483	3·4	15·2	96	
·51 „	·578	4·1	15·2	94	Twilight.
3.11 „	·638	3·0			
·30 „	·688	2·6	15·3	94	Daylight.
·50 „	·740	2·6	15·5	96	
4.30 „	·860	3·0	15·2	96	Clouds.
·34 „	·760	New zero.			
5.27 „	·923	3·1	15·2	96	
·43 „	·962	2·4	15·5	96	Clouds.
6.5 „	4·00	New zero.	15·6	94	
·35 „	·100	2·7	16·2	96	
7.5 „	·210	3·7	16·3	96	
·35 „			16·7	96	
8.0 „	·330	2·2	17·7	93	Brighter.
·35 „			23·6	80	
9.0 „	·470	2·3	20·1	77	Clouds. Watered
·30 „	·550	2·7	20·3	76	about 9.15.
10.0 „	·630	2·7	20·9	74	
·30 „	·720	3·0	23·4	69	Sun and cloud.
11.12 „	·870	3·6	22·6	66	Dull.
·40 „	·980	3·9	22·2	69	
·58 „	5·060	4·4	24·8	64	Sun and cloud. No blinds.
12.17 p.m.	5·110	2·6	24·1	61	Duller.
.21 „					Syringed leaves.
.23·5 „	·150	6·2	23·8	66	
·33 „	·178	2·9	23·1	68	
·43 „	·240	6·2	22·1	68	

EXPERIMENT XVIII (*continued*).

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	μ per min.			
12.53 p.m.	5·300	6·0	21·7	69	
1.3 ,,	·353	5·3			
·23 ,,	4·550	New zero.	21·3	68	
3.0 ,,	5·030	4·9	18·7	72	
5.0 ,,	·570	4·5	17·9	84	
7.0 ,,	6·090	4·3	18·8	87	
8.0 ,,	·370	4·7	16·8	84	
9.0 ,,	·680	5·2	15·2	90	
10.0 ,,	·930	4·2	15·1	91	
11.0 ,,	7·010	1·3	14·8	91	
12.0 ,,	·120	1·8	14·2	94	
July 15.					
1.0 a.m.	7·200	1·3	14·0	96	
2.0 ,,	·380	3·0	14·1	94	
·30 ,,	·500	4·0	14·0	95	
3.0 ,,	·630	4·3	13·9	96	
6.15 ,,	8·640	5·1	18·1	91	Bright morning.
9.40 ,,	9·200	2·7	25·9	64	
2.15 p.m.	10·260	3·8	23·0	68	Cloudy.
4.25 ,,	·710	3·4	21·6	71	
5.0 ,,	·835	3·6	21·9	68	
8.15 ,,	12·790	10·0	17·7	88	
·20 ,,	0·450	New zero.	16·0	90	
10.20 ,,	·960	4·3			

EXPERIMENT XIX.—July 16, 1893. V7.

Time.	Reading.	Rate.	Temp. C°.	Psychr.	
	mm.	per min.			
9.5 a.m.	3·000				
11.0 ,,	·310	2·7	19·4	80	
1.0 p.m.	·670	3·0	17·8	86	Rain.
3.30 ,,	·955	1·9	21·9	74	Sun and cloud.
5.35 ,,	4·265	2·5	19·1	77	Cloud.
10.0 ,,	6·815	9·6	14·2	93	

The fruit was measured again on July 20, when it was 176 mm. in vertical diameter. It measured 113 on July 12.

Fig. 8 is a good example of true growth (i.e. continuous increase without shrinkings), in which the curve of relative humidity is obviously of the same general character as the growth curve.

Fig. 9 shows a steady rate of growth at an intensity less than the rates at 1 p.m. and 6–9 p.m. in Fig. 8. The brighter weather at noon is clearly represented in the parallel disturbance of the two curves. On the other hand the fall in growth rate at 2.37 is not accounted for by the state of the air.

Fig. 10 shows a fairly uniform rate of growth at night—and a rise in rate in the morning in spite of the increased dryness of the air. This rise I believe to be due to the plant having been watered at 9.15. The disturbance in the curves at noon is due partly to alternations of sun and cloud and partly to the syringing of the leaves. There is no marked evening rise in spite of the rise in the humidity curve.

Fig. 11 is chiefly remarkable for the nocturnal fall in growth. The nocturnal temperature fell lower than usual; but this will not account for the fall, since between 1 a.m. and 3 a.m. the curve rises with a constant low temperature.

In none of the above-described figures is there any evidence of the change from night to daylight producing an effect.

Exp. 18 is given as a whole in Fig. 12, where the rate of growth is calculated for intervals of three hours, and expressed as 0·001 mm. per minute.

Omitting the violent temporary rise on the evening of the 15th, it is clear that the rate of growth is gradually sinking. In spite of considerable irregularity, the temporary evening rise is fairly visible, as is the fact that the rate of growth is on the whole stronger by day than by night. The difference, however, is not marked: in the following table the rate of growth has been calculated for the night and day, taking the nearest available hours to 6 p.m. and 4 a.m.; that is a night of 10 hours and a day of 14 hours.

			mm. 0·01 per hour.
July	12	6 p.m.	
			43
,,	13	4 a.m.	
			37
,,	,,	6 p.m.	
			26
,,	14	3.50 a.m.	
			20
,,	,,	5 p.m.	
			21
,,	15	3 a.m.	
			23
,,	,,	5 p.m.	

If this series of readings is graphically represented it will be found that the fall and rise in the rate of growth is so nearly uniform that it is impossible to distinguish night from day.

Observations on the gourd V7 were continued by my friend Mr. Tansley of Trinity College, to whom I am much indebted for a series of careful records. With regard to the comparison of night and day, Mr. Tansley made a few observations between July 23 and 26, which show a clear increase of growth in the night.

	Growth.	Time.	Rate.
	mm.		0·01 mm. per hour.
July 23. 10 a.m. to 10 p.m.	1·960	12 hours	16·3
„ 23–24. 10 p.m. to 10 a.m.	2·950	12 „	24·6
„ 24. 10 a.m. to 10 p.m.	1·140	12 „	9·5
„ 25. 6.40 a.m. to 9 p.m.	1·235	14·3 „	8·6
„ 25–26. 9 p.m. to 6.40 a.m.	1·565	9·7 „	16·1
„ 26. 7.40 a.m. to 8 p.m.	1·035	12·3 „	8·4

The results are interesting in comparison with mine of July 12–15, because at that time the gourd was constantly increasing in diameter, whereas at the end of July it showed the daily shrinking, which has been previously noted in V2 and other fruits. Thus on July 27 the fruit shrunk between 2.30 and 5 in the afternoon; and on 28th between 3.36 and 4.35. On August 2 the shrinkage was so great throughout the day that at 7 p.m. it was smaller by 0·21 mm. than it had been at 7 a.m. Between 7 p.m. and 7.13 a.m. on August 3 it increased 0·420.

The following figures of Mr. Tansley's show the effect of syringing the leaves and soil; we get the same rapid disturbance accompanying a general temporary increase in rate that is shown in Exp. 18.

EXPERIMENT XX.—July 29, 1893. V7.

Time.	Rate.	Reading.	Temp. C°.	Psychr.	Remarks.
	μ per min.	mm.			
7.3 a.m.		11·805	19·1	83	Overcast morning.
10.0 „	+0·5	·898	20·4	82	Rain.
·35 „	0·8	12·003	19·9	88	
·42 „	0·7	·008	19·9	88	Syringed leaves.
·52 „	1·2	·020	19·6	96	Heavy Rain.
·57 „	0·4	·022	19·4	98	
11.1 „	0·5	·024	19·7	95	
·4 „	1·6	·029	19·5	95	
·6½ „	1·2	·032	19·5	95	
·11 „	0·1	·037	19·6	95	
·50 „	0·8	·069	19·7	91	
12.24 p.m.	0·8	·098	19·6	90	Clouded. Rain
1.10 „	0·5	·124	20·3	80	stopped.
2.57 „	0	·123	23·7	75	Pale sun.
3.24 „	−0·2	·117	23·8	75	Cloud.
4.5 „	−0·2	·110	22·9	72	
·38 „	+0·7	·136	20·8	84	Watered.
10.25 „	0·8	·425	16·9	97	

EXPERIMENT XXI.—Aug. 2, 1893. V7.

Time.	Rate.	Reading.	Temp. C°.	Psychr.	
		mm.			
7.0 a.m.		1·21	20·3	90	Dull morning.
9.15 „	−1·4	1·02	26·4	73	
10.20 „	−2·1	0·883	26·3	65	
·55 „	−0·2	·877	24·3	66	Sun and cloud.
11.40 „	−0·2	·870	27·5	61	
12.22 p.m.	−1·3	·813	27·2	61	
1.10 „	−1·5	·740	30·1	59	Sun bright.
·38 „	2·2	·803	24·5	76	1.20, leaves and soil
·41 „	4·0	·815	24·3	76	thoroughly syring-
·43 „	9·0	·833	23·6	73	ed with garden
·45 „	9·5	·852	23·3	70	syringe. 1.25,
·47 „	9·0	·870	23·0	69	cloud.
·49 „	7·5	·885	22·7	70	
·52 „	8·3	·910	22·4	72	
2.12 „	6·75	1·045	22·8	74	
·16 „	4·2	1·062	23·5	78	Hot sun on fruit.
·20 „		·082	23·9	79	
·25 „	1·8	·091	25·5	79	
·28 „	0·6	·093	26·0	76	Cloud.
·32 „	0	·093			Sun.
·34 „	−1·5	·090			Cloud and sun.
3.27 „	−1·3	1·020	24·3	71	Cloud and sun.
4.10 „	−0·9	0·980	24·4	65	Sun on fruit.
·15 „	−0·2	0·970	25·5	70	Sun on fruit and on thermometer.
7.0 „	0·1	1·000	22·1	84	Clear evening.
10.20 „	1·4	1·280	17·1	91	

The figures in Exp. 21 show the much greater effect produced by a thorough syringing of the leaves and soil. On August 2, when the experiment was made, the shrinking of the fruit had, as already noticed, greatly increased. The positive growth due to syringing is quite temporary, and when it is over the fruit begins to shrink at about the same rate which held good before the watering.

General Conclusions.

1. Increase in size or in weight is either continuous or is interrupted by periods of loss in weight or shrinkage in diameter as the case may be.

2. A rapidly growing fruit shows an increase in weight of 0·1 gram per minute. In diameter of 0·01 mm. per minute.

3. When diminution in weight or size is proceeding rapidly, the fruit shows a loss of 0·1 gram per minute: or a shrinkage at the rate of 0·01 mm. per minute.

4. Variations in the rate of growth are chiefly dependent on the hygrometric condition of the atmosphere. Increased relative humidity causes increased growth and *vice versa* [1].

5. No. 4 is true not only in cases where the increase of the fruit is continuous, but also for the cases where growth is interrupted by periods of diminution in size or weight. Thus increase may be converted into decrease when the air becomes dry, and this may again give place to increase when the air becomes more humid.

6. The effects noted in No. 4 and No. 5 probably depend not on the transpiration of the fruit but of the leaves. This view accords with the conclusion (No. 7) that:—

7. Syringing the leaves and watering the soil cause a rapid increase in growth.

[1] This result agrees, generally speaking, with G. Kraus' classical researches on the distribution of water in plants, to which reference has already been made.

8. There is no evidence that the change from night to daylight or *vice versa* has any effect *per se*.

9. The curve of growth shows a minimum in the afternoon followed by a rapid rise towards evening.

10. The evening rise is followed by a fall in the curve as the night proceeds.

11. The rate of growth is more uniform by night than by day.

It would be of interest to inquire how far the facts here recorded find a parallel in the growth of internodes; but to this point I can only refer in the briefest manner.

Mulder, as quoted by Sachs [1], observed the growth of the leaf of *Urania speciosa*: Sachs points out that Mulder's records give a remarkable opposition between the curves of growth and temperature,—one sinking as the other rises. This is paralleled by my observations on the change of weight in the fruit of *Cucurbita*, and is no doubt a reflection of a similar change in conditions, i. e. variation in the relative humidity of the air.

Sachs also gives [2] the results obtained by W. de Vriese in 1847. The flower-stem of *Agave americana* was found to show either a complete cessation of growth, or an actual shortening, during the morning. Here again the phenomenon clearly depended on the relative distribution of water in the leaves and stem. Thus on two occasions, when owing to clouds or rain the transpiration must have been small, a small amount of growth took place in the morning. More recently Godlewski [3] has shown that in the epicotyl of *Phaseolus* any great diminution in the dampness of the air produces a sudden but temporary slowing in growth, while a temporary rise in growth-rate follows an increase in humidity. In some of my experiments this temporariness of the effect of changes in the hygroscopic condition of the air has been clearly marked.

[1] Arbeiten, i. p. 174.

[2] loc. cit. p. 183.

[3] Anzeiger der Akad. zu Krakau, 1890. I quote from the Bot. Centralblatt, 47, p. 307.

In conclusion I wish to express my thanks to those who have helped me by making observations. I would especially mention Mr. Lynch, the Curator of the Botanic Garden, who was untiring in giving me his valuable help. I am also much indebted to Miss Pertz, Mr. Tansley, Mr. de Havilland, as well as to Mr. Lamb of the Botanic Garden, and to my Laboratory assistant Mr. Elborn.

EXPLANATION OF THE FIGURES IN PLATES XXII AND XXIII.

Illustrating Mr. F. Darwin's paper on the Growth of the Fruit of *Cucurbita*.

Plate XXII.—Figs. 1–4 inclusive represent the rate of change in weight expressed in milligrams per minute. The broken line represents the temperature.

Fig. 1 gives Experiment III.
Fig. 2 „ „ IV.
Fig. 3 „ „ V.
Fig. 4 „ „ VIII.

Figs. 5, Plate XXII, to Fig. 12, Plate XXIII, represent the rate of change in the diameter of the fruit, expressed in μ (0·001 mm.) per minute. The broken line represents relative humidity in all except Fig. 5, where it represents temperature.

Fig. 5 gives Experiment XI.
Fig. 6 „ „ XVI.
Fig. 7 „ „ XVII.
Fig. 8 „ „ XVIII.
Fig. 9 „ „ „
Fig. 10 „ „ „
Fig. 11 „ „ „
Fig. 12 „ „ „

Figs. 8–11 give the detail of a continuous series of observation from the morning of July 12 to the morning of July 15.

Fig. 12 gives Experiment XVIII as a whole, including observations up to the evening of July 15.

Fig. 1.

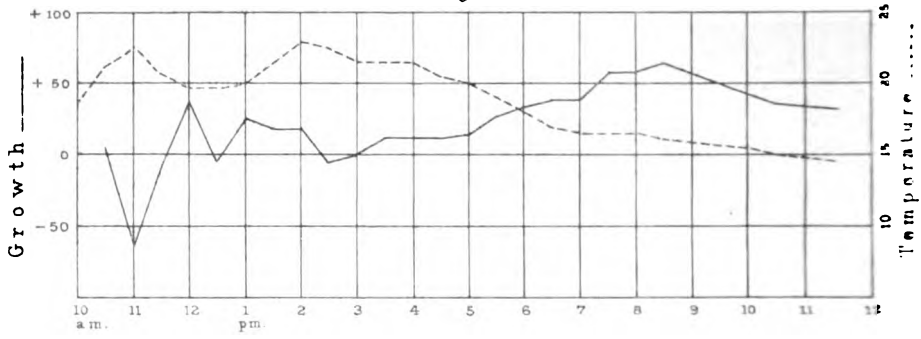


Fig. 3.

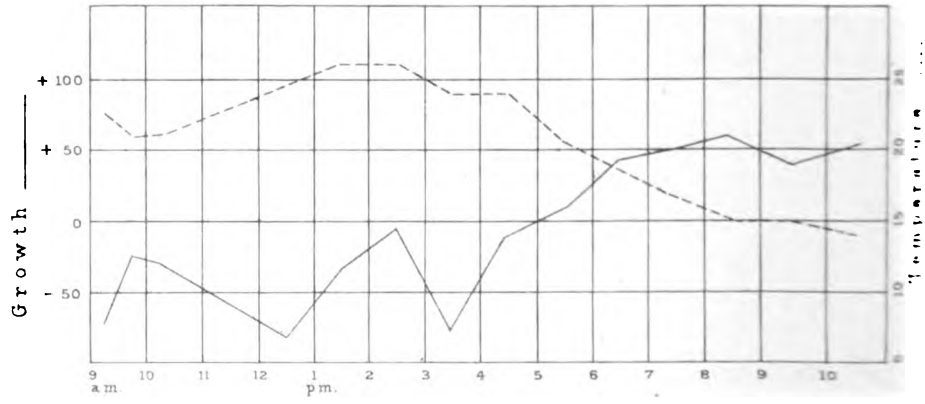
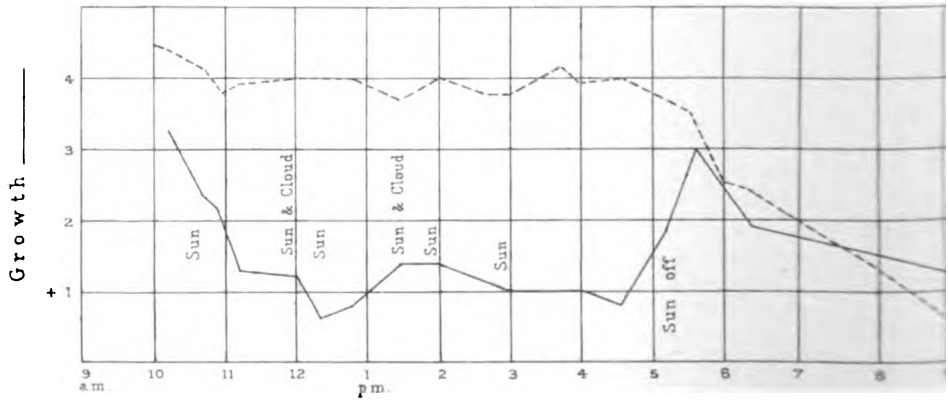


Fig. 5.



DARWIN.— GROWTH OF CUCURBITA.

Fig. 2.

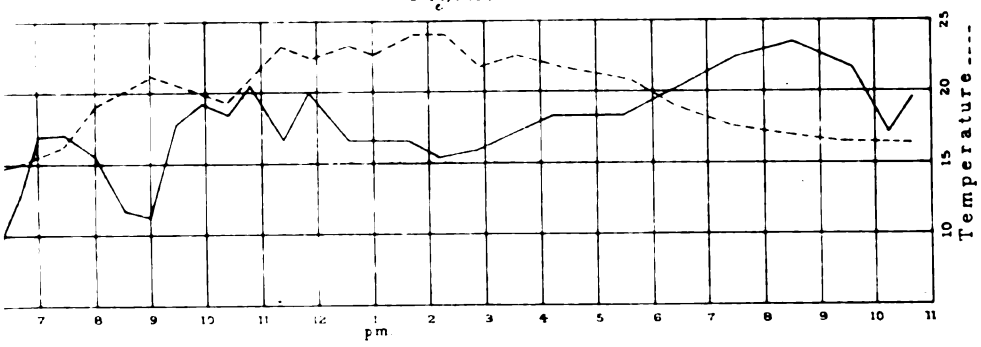


Fig. 4

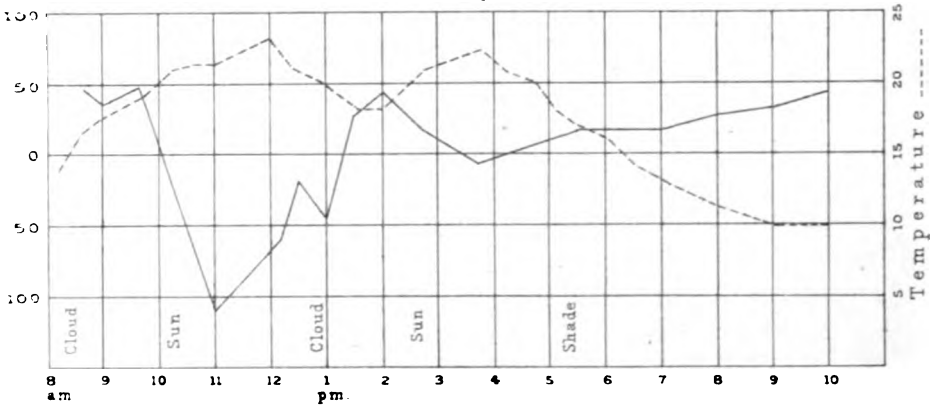


Fig. 6.

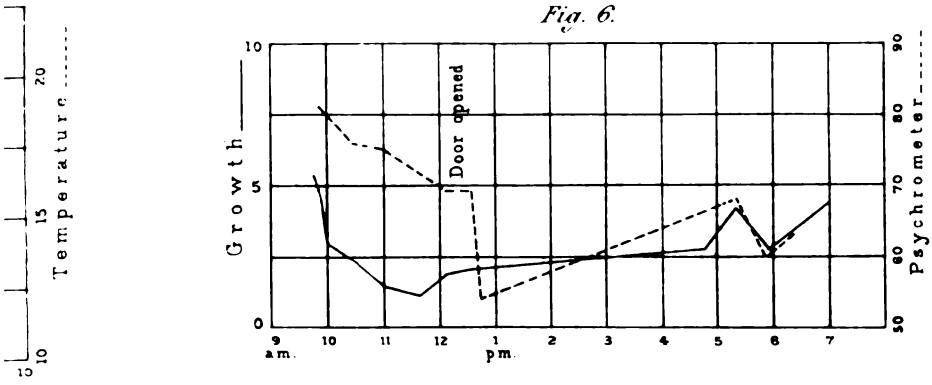


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Fig. 7.

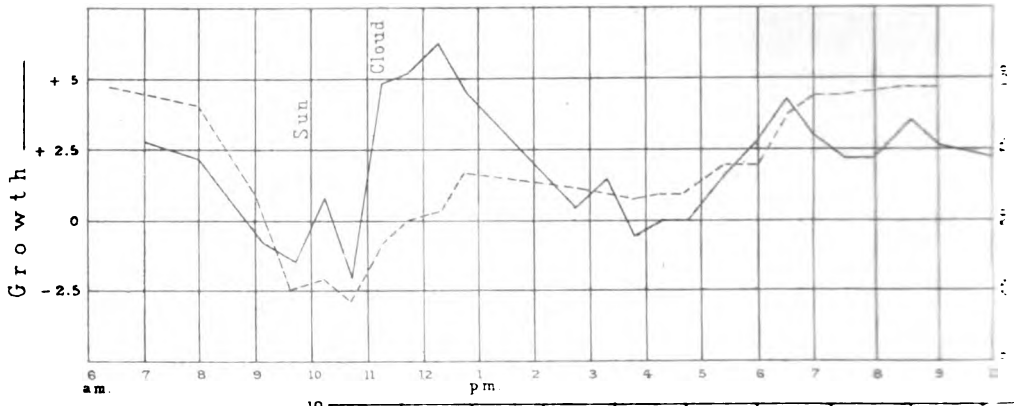


Fig. 9.

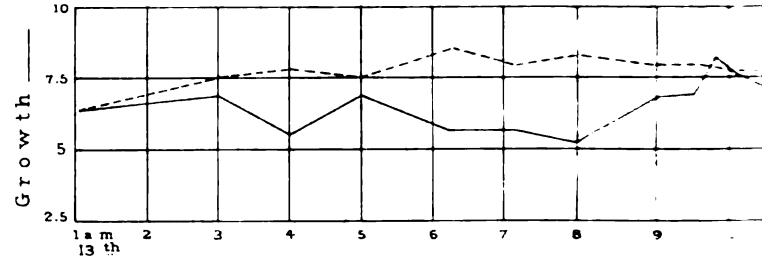


Fig. 10.

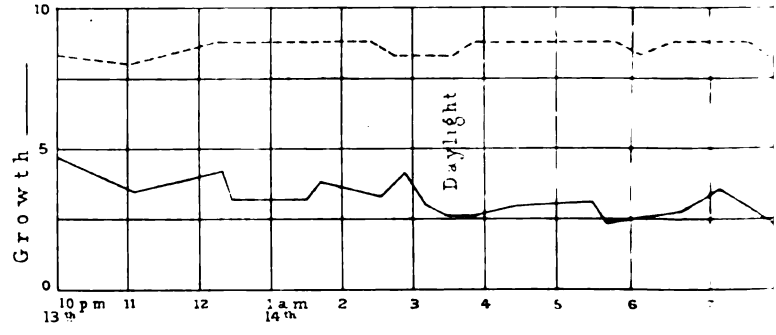
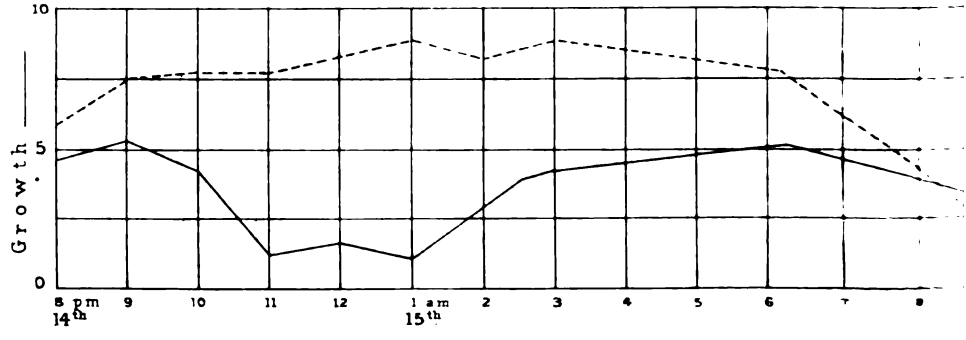


Fig. 11.



DARWIN. — GROWTH OF CUCURBITA.

Fig. 8.

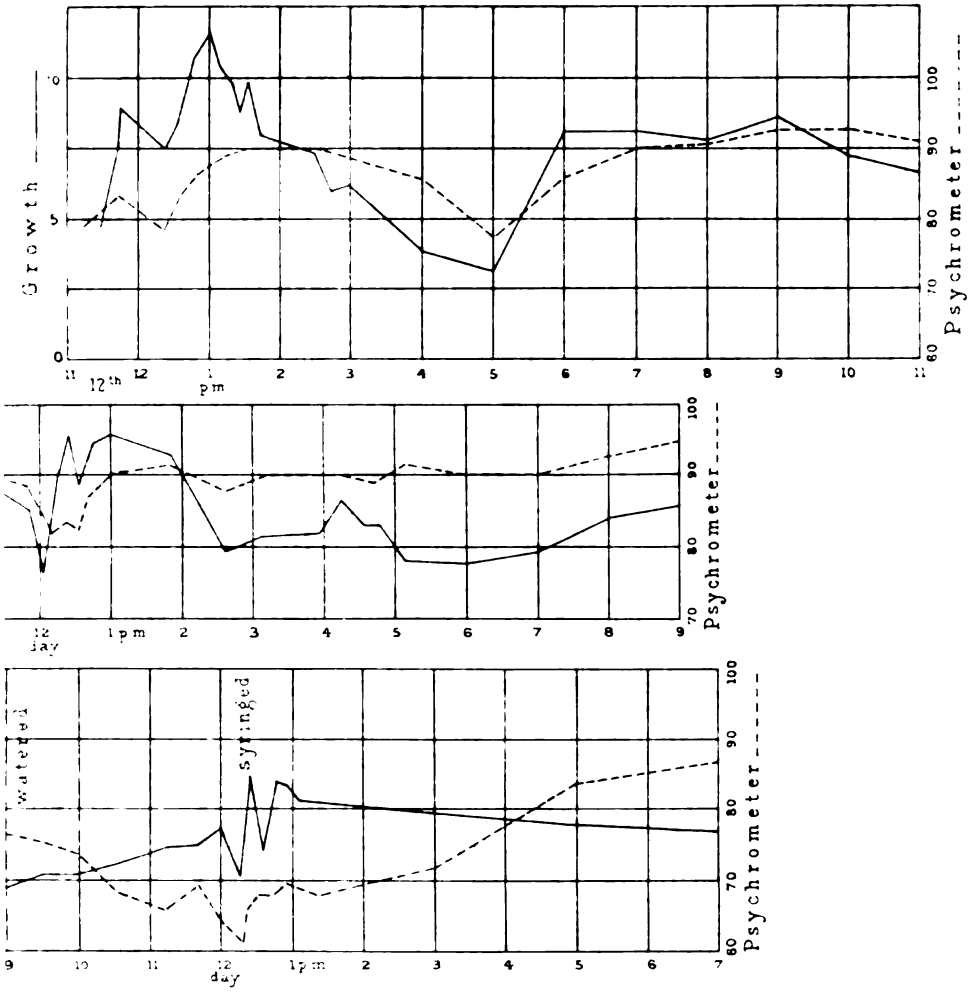
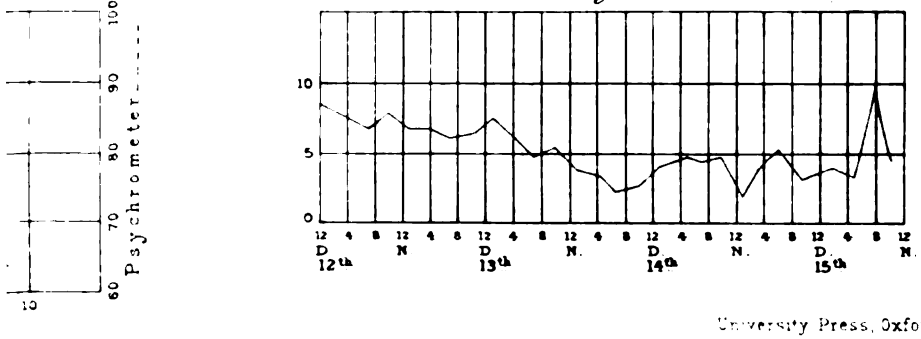


Fig. 12.



University Press, Oxford

On Nuclear Division in the Hymenomycetes.

BY

HAROLD WAGER, F.L.S.,

Lecturer on Botany in the Yorkshire College, Leeds.

With Plates XXIV, XXV, and XXVI.

INTRODUCTION.

THE question of the structure and division of the nuclei in the lower plants is one of considerable interest to histologists, and has attracted the attention of numerous observers in recent years. The results obtained, however, leave much to be desired, especially as regards the nuclei of the Fungi. It is a matter of importance to determine to what extent the process of nuclear division in these plants corresponds with the indirect division, or karyokinesis, which occurs in the cells of the higher plants, the complexity and regularity of which indicate that the nucleus plays an important part in the life-history of the cell. The various observations which have been made upon the nuclei of the Fungi up to the present time, with one exception, tend to show that although the process of division resembles in many respects that which takes place in the higher plants, it is very much simpler, and does not include some of the details which are considered by histologists to be of great importance in the process of nuclear division.

In the present paper an attempt is made to throw light upon this subject by a description of some observations which

[Annals of Botany, Vol. VII. No. XXVIII. December, 1893.]

have been made on the nuclei of the Hymenomycetes, a preliminary account of which has already been published [1].

The observations were made upon two species of the Agaricineae—*Agaricus* (*Stropharia*) *stercorarius*, and *Agaricus* (*Amanita*) *muscarius*—and were confined entirely to the structure and division of the nuclei in the basidia, which, on account of their large size as compared with the nuclei of the hyphae, are much more favourable objects for the observation, a very difficult one in this case, of nuclear division.

Literature.

Until very recently observations upon the nuclei of the Hymenomycetes were few in number, and confined mainly to the determination of their presence or absence in the various cells of these plants. Very little was known of their structure and practically nothing concerning their division. De Bary states [2]:—'It may be assumed that there is a nucleus always present (in the basidia) though in the smaller forms it has been looked for in vain up to the present time. Where it has been observed, as in *Dacryomyces*, *Calocera*, *Corticium calceum*, and especially in the basidia of *Corticium amorphum* . . . it is a spherical feebly refringent body (perhaps the nucleolus) lying near the centre of the cell. It is not to be seen in the early stages of the basidium and it disappears when the formation of sterigmata commences.' Strasburger [3] also is only able to state that nuclei exist in the cells of several of the Agaricineae, and that the young basidium contains a single nucleus, which divides into two at the time when the sterigmata begin to form, and then further divides until as many as eight may be observed in the basidium.

A considerable advance in our knowledge of these plants has been made in more recent times by Rosenvinge [4], who

[1] On the Nuclei of the Hymenomycetes, Annals of Botany, Vol. VI. p. 146, 1892; British Association Report for 1891, p. 700.

[2] Comp. Morph. and Biology of the Fungi, &c., English edition, p. 64.

[3] Botanisches Practicum (1884, p. 324 and 427); (1887, p. 301 and 433).

[4] Sur les Noyaux des Hymenomycetes, Ann. des Sci. Nat., Bot., Sér. VII, tome 3, 1886.

states, in a paper of considerable interest, that he has examined thirty-five species of Hymenomycetes and finds only a single nucleus in the young basidia. The cells of the hyphae, on the other hand, contain one or more nuclei. The largest nuclei were found in species of *Boletus* and *Amanita*. Sometimes the nucleus has a vesicular structure, the chromatin forming a peripheral layer on the wall of the nucleus (*Amanita*, *Boletus*). A nucleolus is very rare (*Amanita muscarius*). If the nuclei are very small, they are very dense and refringent. In *Tricholoma virgatum*, the nuclei of the young basidia are granular; they appear to have a reticulate structure, but do not possess a nucleolus. In species of *Amanita* (*A. vaginata* and *A. porphyria*) the nucleus resembles a vesicle with a firm wall, thickened on the inferior side or on two opposite sides. But this appearance of the wall is due to the chromatin which is disposed on the periphery of the nucleus, against the wall of the nuclear sac, which can be distinguished sometimes when the nucleus is a little contracted. This nucleus always contains a nucleolus, sometimes free in the centre of nucleus, sometimes in contact with the periphery. When the nucleolus is free it seems to indicate that the cavity of the nucleus is not a vacuole, but that it contains a non-colourable substance, the nucleo-hyaloplasm of Strasburger. Rosenvinge was not able to make out any of the details of division. Indications of indirect division of the nucleus were found in *Tricholoma virgatum*, but he was not able to follow it out. The nucleus of the basidium divides first of all into two, then into four, or eight. In *Amanita* the sterigmata commence to form after the secondary division of the nuclei into four; in *Tricholoma* after the first, but before the second nuclear division is accomplished. The nuclei pass from the basidia into the spores, each one of which receives one or two. In passing, if the nucleus is larger than the diameter of the sterigma, it accommodates its form to the latter and becomes elongated.

No further observations were made upon this subject, so far as I know, until the year 1891, when I communicated to Section D of the British Association a preliminary account of my observa-

tions upon the structure and division of the nuclei in the basidia of *Agaricus* (*Stropharia*) *stercorarius*[1], in which I pointed out that not only did the nucleus possess a structure closely resembling that of the higher plants, but that the nuclear division more nearly resembles that of the latter, inasmuch as a distinct equatorial plate, and apparently a spindle-figure, were produced.

I have briefly referred to this paper here because Rosen[2] has more recently published a description of some observations upon nuclear division among the Basidiomycetes, in species other than those I have examined, which do not, if I understand him rightly, at all agree with mine. It will be useful to briefly summarize his results. He observed many different species, but as they all agree in their chief details, he confines himself mainly to a description of *Lepiota* (*Armillaria*) *mucida*.

The young basidia contain two or more nuclei, generally arranged in pairs. The mature basidium contains a single nucleus. This basidial nucleus arises by the repeated fusion of the smaller nuclei originally contained in the basidium—six or eight probably. From an examination of *Psalliota campestris* he thinks it possible that the majority of these small nuclei may be produced in the basidium.

The single nucleus of the mature basidium is at first dense, and allows only faint indications of a nuclear network to be seen, in close proximity to the nucleolus. The nucleus soon increases in size, however, and then it is seen that the chromatin is restricted to a long coiled and crumpled thread. Soon numerous sharp bends appear in it, and finally the thread becomes broken up into segments. These segments collect at last into two little star-shaped heaps, opposite each other, in the wide nuclear cavity; then, the nucleolus becomes dissolved and the nucleus divides into two, without any indication of spindle-figure or connecting threads. In each

[1] Loc. cit.

[2] Studien über die Kerne und die Membranbildung bei Mynomyceten und Pilzen, Cohn's Beiträge zur Biologie der Pflanzen, Bd. VI. p. 259, 1892.

daughter-nucleus a new nucleolus is formed, and very soon a further division of the two nuclei takes place, which appears to be much the same as that just described. Rosen does not give any details as to the precise way in which the final separation of the nucleus into two daughter-nuclei is accomplished, whether by constriction of the primary nuclear membrane, or by its disappearance and the subsequent formation of two new ones, points of sufficient importance, one would have thought, to have been carefully determined.

Gjurasin has recently published a paper upon nuclear division in the asci of *Peziza vesiculosa*[1], which has an important bearing on some of the facts mentioned in this paper. The nucleus of the young ascus consists of a nuclear membrane, chromatin-network, and a large nucleolus. By means of Hermann's safranin-gentian violet, he has been able to colour the network pale blue-violet and the nucleolus ruby red. In the process of division, the nucleus elongates slightly in the direction of the long axis of the ascus; the nuclear network becomes granular, and now colours red. A spindle-figure is then formed, the threads stretching from the protoplasm at both ends of the now barrel-shaped nucleus, to the middle of the latter. At each pole of the spindle, protoplasmic radiations can be made out, but no attraction-spheres. The granules of the nuclear network pass to the equator of the spindle, divide here into two halves, and move very quickly, to the two poles of the spindle. The nucleus elongates still more, and the radiating striae gradually disappear. The nucleolus has remained unchanged all this time, but now becomes slightly elongated. The nuclear membrane finally disappears and the nucleolus comes to lie directly in the protoplasm. The two daughter-nuclei become surrounded with new nuclear membranes, and the nuclear network again colours blue-violet. Nucleoli make their appearance in the daughter-nuclei, as very small bodies whose size continually increases. As the nucleoli of the daughter-nuclei increase in

[1] Ueber die Kerntheilung in den Schläuchen von *Peziza vesiculosa*, Bulliard, Berichte der Deutsch. Bot. Gesellschaft, Bd. XI. p. 113, 1893.

size, the original nucleolus in the protoplasm becomes smaller, until at last it completely disappears. The two daughter-nuclei divide again in the same way, as do the four nuclei produced by this second division, until the eight nuclei are produced.

I will now give an account of my own observations.

METHODS.

The following is a short account of the methods employed in this investigation.

The fungi were cut up into short pieces, as soon as possible after they were collected, which were placed in a saturated solution of corrosive sublimate in which they were left for about twelve hours; they were then washed well in water and gradually brought into methylated spirit through 30, 50, and 70 per cent. spirit. They were next passed successively through absolute alcohol, xylol, and paraffin, and embedded in the usual way in blocks. The pieces of tissue were not left in the paraffin longer than was sufficient to allow the complete penetration of the latter, which was kept at as low a temperature as possible. Moderately thin ribbons of sections were cut by the microtome. These were cut into short lengths, flattened by dropping them carefully on to the surface of warm water and were then taken up on glass slips and allowed to dry. By this means the sections became attached sufficiently firmly to the slip to render cement unnecessary. The slides were then warmed just enough to melt the paraffin, and placed at once in turpentine or xylol to remove the paraffin. They were then brought into absolute alcohol to get rid of the turpentine or xylol, and were finally placed, ready for staining, in methylated spirit.

Numerous staining methods were employed and it was found that all the ordinary stains such as haematoxylin, carmine, safranin, &c., coloured the nuclei more or less deeply, but it was found that none of these stains brought out the structure of the nucleus distinctly; moreover, it was impossible by means of them to get any clear indication of nuclear

division. I am indebted to Professor Marcus Hartog, however, for a method of staining, not yet published, which gives beautiful results, and I take this opportunity of thanking him for his kindness. I am not at liberty to give here the exact method employed, but it will suffice to state that two stains are used, Mayer's alcoholic solution of carmine and a strong acetic solution of nigrosin. By this method I have been able to demonstrate satisfactorily not only the structure of the nucleus but also the various stages in the process of its division, and I have also obtained a double staining of the nucleus in which the nucleolus becomes bluish-red, the network and nuclear membrane blue. The cytoplasm at the same time becomes red or reddish-blue.

The sections were examined with the apochromatic object-glasses of Zeiss, of which I have used the 1·3 aper. 2 mm., 1·4 aper. 2 mm., and 1·3 aper. 1·5 mm. with oculars 8, 12, and 18, and a sub-stage condenser of high angular aperture made especially for high-power lenses by Swift. It is necessary to pay considerable attention to the illumination of the object in using these high powers. The best light is obtained from the edge of the flame of a paraffin lamp, with a metal chimney covered with some dead-black material on the inside. This is preferable even to the best daylight.

Structure of the Basidia.

If sections through a moderately young pileus of *Agaricus stercorarius* or *A.* (*Amanita*) *muscarius* be examined, they will be found to exhibit basidia in all stages of development. The basidia of the two species differ considerably in appearance owing to difference in density of their respective protoplasm. In *Agaricus stercorarius* the protoplasm is dense and granular, stains very deeply, and possesses but few or no vacuoles. The protoplasm in *A. muscarius*, on the other hand, is at no time very dense and, except in the very young stages, is generally full of vacuoles. The latter species is therefore a much more favourable object for the examination of the details of nuclear

division, than the former, in which the density of the protoplasm somewhat obscures this process.

A basidium first appears as a small club-shaped branch projecting from the sub-hymenial layer. A quantity of protoplasm passes into it from the hypha upon which it is borne. This may completely fill the basidium, or vacuoles may be present, especially at first. At a very early stage in the development of the basidium, two or more nuclei make their appearance in it. These appear to pass over from the hypha along with the protoplasm. The number of nuclei which thus make their appearance is two in *A. stercorarius*, two or three in *A. muscarius* and, according to Rosen, as many as six or eight in some other species. These nuclei are larger than the nuclei of the hyphae, and they may have arisen, therefore, by fusion of two or more of the latter during their entry into the basidium, or their larger size may be due to the superior nourishment to be obtained in the protoplasm of the basidium, as compared with that to be obtained in the hyphae.

The structure of these nuclei can be very well made out at this stage (Figs. 1, 2, 31, 32, 33). Each nucleus possesses a large deeply stained nucleolus, a very clearly defined nuclear network, in which a granular structure could be made out, especially in *A. muscarius*, and a well-marked nuclear membrane.

As the basidia increase in size, the primary nuclei, as we may call them, fuse together into a single large nucleus. I have been able to obtain sections showing all the stages of this fusion in *A. stercorarius*, so far as this can be ascertained in dead, stained specimens. Absolute proof that this fusion takes place depends perhaps upon the observation of living material, but this is impossible at present owing to the difficulty of observing the nuclei in the living state. The details of the fusion, as they were observed in stained specimens of *A. stercorarius*, are as follows:—The two nuclei gradually approach each other until they come into such close contact that a flattening of the two is produced where they touch each other (Figs. 3 and 4). A dumb-bell shaped structure is thus produced. The membrane separating the two nuclei now

disappears, and the nuclear network of the one nucleus becomes intermingled with that of the others. An oval-shaped nucleus is thus produced, in which the two nucleoli of its component nuclei can be distinctly seen (Fig. 5). The two nucleoli remain distinct for some time, but eventually they approach each other, come into contact, and finally fuse into a slightly elongate nucleolus which at last becomes perfectly spherical (Figs. 6 and 34). We have then a complete and regular fusion of all the parts of the two nuclei, resulting in the formation of a single large nucleus in the basidium. The basidium now increases largely in size, together with the nucleus. The nucleus takes up a position in the upper expanded portion of the basidium, and is found to occupy at least two-thirds of the diameter of the latter.

It is at this stage that the structure of the nucleus can be most easily made out (Figs. 6 and 34). It consists of a nuclear membrane, nuclear network, and a large nucleolus. The nucleus is very distinctly defined by the nuclear membrane, and stands out boldly in the protoplasm, especially in *A. stercorarius*. The nuclear network is made up of a thread or threads, which are coiled into a somewhat loose knot. Rosen [1] finds that the network in the species examined by him consists of a single much coiled thread. The thread consists of a matrix, staining light blue, in which are embedded numerous granules of chromatin. The latter are placed at nearly regular intervals along the thread, and stain deep blue. The nuclear sap between the threads is unstainable.

The nucleolus is very large, and stains deep bluish red: it is by far the most conspicuous object in the basidium, and has been often mistaken, doubtless, by earlier observers for the nucleus itself. It consists generally of a homogeneous mass, but often exhibits a vesicular structure, and occasionally is so full of small, clear spaces, like vacuoles, as to give it almost the appearance of a coarse network. Sometimes the nucleolus appears to be lodged in a distinct cavity in the nucleus. The nuclear network surrounds this cavity, but is not in contact

[1] Loc. cit.

with the nucleolus at any point (see Fig. 19): in many cases I obtained sections in which the nucleolus had become dislodged from this cavity during the process of preparing the section.

Division of the Nucleus.

The nucleus of the basidium divides, first into two, then into four daughter-nuclei. The details of the division are difficult to make out, even with the highest powers of the microscope, and in *Agaricus stercorarius* are also somewhat obscured by the dense protoplasm. By careful staining and examination, however, it is possible to show that the division is distinctly karyokinetic, and that it corresponds in all its details with that observed in the higher plants and animals. There appear to be some slight differences of detail, in the earlier stages of division, in the two species examined. I will therefore describe them separately.

In *A. stercorarius* the nucleus first of all increases somewhat in size and becomes elongated in the direction of the long axis of the basidium (Fig. 7). The nucleolus takes up a parietal position on the wall of the nucleus at its lower end. The nuclear network appears to become broken up into segments, which stain a little more deeply than before. The nuclear membrane at the upper end of the nucleus becomes indistinct and finally disappears, but the lower portion remains intact for some time longer. The nucleolus now begins to decrease in size, and takes a brighter red stain than formerly. At the same time the protoplasm, in the region of the nucleolus, stains more deeply. The dark blue chromatin-segments, which have been accumulating at the extreme apex of the nucleus as a somewhat homogeneous mass, now seem to disappear, and in their place a group of deeply stained red granules, or short rods, is to be seen (Fig. 8). These have been produced probably by condensation of the chromatin-segments. This change in the nature of the chromatic elements coincides with a further change in the nucleolus, which gradually becomes smaller and loses, at the same time, its capacity for taking up the red stain, and stains only light blue.

The lower portion of the nuclear membrane now becomes indistinct and finally disappears, so that both the nucleolus and the chromatic elements now stand free in the protoplasm. For some time, however, a slightly clearer space can be made out around the nucleolus, which probably represents the old cavity of the nucleus, not yet completely filled up by the dense cytoplasm.

The chromatic elements are extremely small and might easily be mistaken for large protoplasmic granules, especially in badly stained specimens; but as they stain very deeply and of a slightly different colour from the protoplasm, they can be easily distinguished, and are in fact by far the most conspicuous objects in the basidium at this stage. We may regard this group of bright red chromatic elements as the equatorial plate.

The details just described agree in the main with those which take place in *A. muscarius,* but owing to the less dense nature of the protoplasm in the latter, the origin of the group of chromatic elements which forms the equatorial plate, can be traced more easily to the original chromatin-network of the nucleus: we shall find that the formation of these chromatic elements corresponds in all essential details with the formation of the chromatic elements in the higher plants and animals.

The nucleus first of all, as in *A. stercorarius*, increases in size. The chromatin-network then breaks up into a number of slightly curved segments, in which both the original structure of the thread and its behaviour towards the carmine-nigrosin stain are for a time preserved (Fig. 35). The nucleolus remains unchanged during this segmentation of the nuclear thread. The chromatin-segments very soon become shorter and thicker and more sharply and irregularly curved. The original structure of the thread disappears; the chromatin-granules become indistinct and probably fuse together, until at last each segment becomes transformed into a crumpled, homogeneous mass which still stains deep blue (Fig. 36). These irregular masses gradually condense into irregularly shaped, bright, refractive granules (Fig. 37). In this condition

they behave somewhat differently towards stain than before. They tend to take up the red stain, and in consequence become coloured reddish blue instead of deep blue, and are also more intensely stained. Between these granules a few delicate connecting threads, stained light blue, were to be seen. The segments, during their transformation into these granules, become considerably reduced in number, but I was not able to determine if this be due to their fusion. The nuclear membrane at this stage becomes irregular in outline, is less clearly defined from the cytoplasm, and soon afterwards almost completely disappears.

The chromatic elements now group themselves in the middle line, near the apex of the basidium, to form a more or less regular equatorial plate (Fig. 38). They become smaller, brightly refractive, and stain, more intensely than before, a bright red. This renders them very distinct, and differentiates them most clearly from the surrounding cytoplasm, which is itself stained more deeply than before. The nucleolus, meanwhile, becomes very much smaller in size, loses its capacity for taking up the red stain, and instead of the reddish blue which formerly distinguished it, is now coloured only light blue, and is not, as in the earlier stages, the most conspicuous object in the basidium.

Finally the nuclear membrane completely disappears, and the nuclear elements come to lie quite freely in the protoplasm. The disappearance of the nucleoli is probably due to their solution in the nuclear sap, as is the case, according to Strasburger[1], in the higher plants. At the time when the nuclear membrane disappears, the cytoplasm becomes more deeply stained. This is probably due to the escape of a portion of the dissolved nucleolar substance into it. From the fact that the chromosomes begin to stain red at the time of the disappearance of the nucleoli, it would further appear that the former can take up nucleolar substance from the nuclear sap, and as fast as the nucleoli disappear the chromatic elements become more deeply stained red. It is impossible

[1] Controversen der indirecten Kerntheilung, p. 8, 1884, and later works.

to say definitely that the nucleolar substance is taken up in such a way, but it seems to me that this explanation is a legitimate one, and best fits the facts observed.

The Nuclear Spindle.

The formation of a nuclear spindle takes place soon after the disappearance of the nuclear membrane and the formation of the equatorial plate (Figs. 9–12 and 39–41). It is placed transversely in the basidium, and consists of a few very fine and delicate threads. These diverge only very slightly from one another, so that the spindle appears very narrow. It can be most easily observed in *Agaricus muscarius*, but is also distinctly visible in *A. stercorarius*. At each pole of the spindle, can be seen, in favourable specimens, especially in *A. stercorarius*, two very minute, deeply-stained granules (Figs. 9–12). These occupy a position similar to that of the centrosomes of the attractive spheres, which are to be found, as Guignard has shown, in the cells of the higher plants[1], but no trace of the clear border with circular outline, or the radiating striae, described by this author, were to be seen. It is perhaps not advisable, therefore, at present to speak of these bodies definitely as centrosomes, but their presence here, taken in conjunction with Gjurasin's discovery of radiating striae at the poles of the spindle in the ascus of *Peziza*, renders it probable that with improved methods of staining and observation, these bodies will be found to belong to definite attraction-spheres.

Formation of Daughter-Nuclei.

The chromatic elements now begin to divide into two groups which move towards the poles of the spindle. Previously to the separation, a longitudinal splitting of the chromatic elements was not observed. This may have been due to their excessive smallness, which renders the deter-

[1] Sur l'existence des 'sphères attractives' dans les cellules végétales—Compt. Rend. Acad. des Sci., 1891. Nouvelles études sur la Fécondation—Ann. des Sci., Bot., Sér. VII. tome 14, 1891.

mination of such a point a matter of great difficulty. It is not safe to affirm, therefore, that it does not occur.

The nucleolus has persisted all this time, and is still visible as a small body, which stains light blue (Figs. 9, 11, 39), but it now begins to disappear entirely, and in the majority of cases has completely vanished, by the time the chromatic elements have reached the poles of the spindle. Cases occur, however, in the division of the daughter-nuclei, as will be seen later on (Fig. 23), where the nucleoli persist until the chromatic elements have reached the poles of the spindle and are in a state of fusion. In any case they disappear before the complete formation of the daughter-nuclei.

When the chromatic elements arrive at the poles of the spindle, they begin to fuse together into two irregular masses which stain bright red (Figs. 13–16.) Some, one or two, threads of the spindle-figure remain for some time connecting them together (Fig. 42), but eventually they disappear. The two masses thus formed are generally in close contact with the wall of the basidium; in some cases they even produce a slight bulging out of the latter.

The subsequent changes which take place in the formation of the daughter-nuclei are slightly different in the two species.

In *A. muscarius* the new membranes for the daughter-nuclei now appear. The chromatin-mass in each remains in a parietal position, near the wall of the basidium. Radiating from this red mass in each nucleus, a few fine threads can be made out (Fig. 43), forming a faintly-stained network. The nuclei then increase in size. The chromatin-masses become apparently transformed into nucleoli, which gradually increase in size, whilst the network becomes more and more distinctly differentiated, until, at last, the two nuclei come to resemble the parent nucleus, both in structure and staining properties (Fig. 44).

In *A. stercorarius* the chromatin-mass appears to be transformed at once into the nucleolus, and for a time there is a clear space between the nucleolus and the nuclear membrane. The outline of each nucleus is at this stage very

irregular, and presents a characteristic appearance (Fig. 17). Soon, however, a faintly-stained network appears. This becomes more and more distinct and stains deep blue. The outline of each nucleus becomes more regular, and finally the two nuclei arrive at the stage in which they in every way resemble the parent nucleus. The two nuclei thus formed are placed side by side at the apex of the basidium, and take up nearly the whole of its diameter (Fig. 19).

As the daughter-nuclei gradually increase in size and assume the form and structure of the parent nucleus, the nucleoli also become larger, and both nucleoli and network soon have the same capacity for staining as in the parent nucleus. At the same time the protoplasm loses its capacity for intense staining. It is probable, therefore, that the increase in size of the nucleolus is due to the absorption by it of nucleolar substance from the cytoplasm.

Behaviour of the Nuclei towards the Red and Blue Stains.

The changes which take place in the nucleolus and chromatic elements, as regards their capacity for taking up the red and blue stains, are very interesting, especially in the light of the recent researches of Auerbach[1], Schottländer[2], Rosen[3], Strasburger[4], and Zacharias[5], upon this phenomenon. Moreover, they appear to me to throw some light upon the much-debated question of the nourishment of the chromatic elements by the dissolved nucleolar substance. In connexion with this question, Went[6] has already pointed out that, on

[1] Ueber einen sexuellen Gegensatz in der Chromatophilie der Keimsubstanzen. Sitzungsber. der K. preuss. Akad der Wiss. zu Berlin, 1891.

[2] Beiträge zur Kenntniss des Zellkerns und der Sexualzellen bei Kryptogamen. Cohn's Beiträge zur Biologie der Pflanzen, Bd. VI, 1892.

[3] Beiträge zur Kenntniss der Pflanzenzellen I. Cohn's Beiträge zur Biologie der Pflanzen, Bd. VI, 1892.

[4] Ueber das Verhalten des Pollens, &c., Hist. Beiträge, Heft iv, 1892.

[5] Ueber Chromatophilie. Ber. d. Deutsch. Bot. Gesells. Bd. XI, 1893.

[6] Went, Beobachtungen über Kern- und Zelltheilung, Berichte d. Deutsch. Bot. Gesells. 1887.

staining the nuclei in the embryo-sac of *Narcissus pseudo-narcissus* with fuchsin-iodine green, the nuclear thread in the resting-stage stained blue-green, and the nucleolus red. After the nucleolus had become dissolved in the nuclear sap, the nuclear threads stained violet, and remained so during the metaphase and partly during the anaphase, until at last they again stained only blue-green. This seems clearly to indicate that the dissolved nucleolar substance is taken up into the nuclear threads. Strasburger[1], however, whilst regarding the nucleolus as a storage of reserve substance, does not think it probable that this reserve substance goes to nourish the chromatic threads, but that it is used, in part at least, in the formation of the cell-wall, and accumulates for this purpose in the equatorial region of the dividing nucleus, where the cell-plate is being formed.

Whether this be true or not, the observations of Went and myself, that, as the nucleus becomes dissolved the chromatic elements stain more intensely and of the same red colour as the nucleolus, seem to indicate that the chromatic elements owe this erythrophil reaction, though not necessarily dependent on nutrition, to the dissolved nucleolar substance. The observations of Meunier[2] and Moll[3], that the chief part of the chromatic (colourable) substance, in the nuclear fibrils of *Spirogyra*, come from the nucleolus, seem also to strengthen this view.

It seems to me possible to modify Strasburger's view to some extent, so as to bring it more into accordance with these observations. May we not, in fact, regard the nuclear threads as carriers of nucleolar substance during the process of nuclear division, and at the same time retain the view that the nucleolar substance is a reserve substance to be used up in the formation of such structures as the cell-wall?

I would suggest that the nuclear threads take up the

[1] Ueber Kern- und Zelltheilung im Pflanzenriche, 1888.

[2] Meunier, Le nucléole des *Spirogyra*. La Cellule, T. III, Fasc. 3, 1888.

[3] Moll, Observations on Karyokinesis in *Spirogyra*. Verhandl. der K. Akad. v. Wetensch. te Amsterdam, Sect. II, D. i, 1893, No. 9. See Bot. Zeit. No. 18, 1893 and Bot. Centralblatt, 1893, Beihefte, p. 407.

dissolved nucleolar substance at some period during the division, and carry it over into the daughter-nuclei, to be given up again later as the nucleoli of the latter.

In those cases where a cell-plate is formed, and where a portion of the nucleolar substance is, according to Strasburger, to be regarded as going to nourish it and to help in its formation, the superabundant nucleolar substance only would be taken up in this way. And this would probably not take place until a somewhat late stage in the division.

Strasburger's[1] observation that the nuclear threads in the stage of metaphase are always cyanophil, and that they only become erythrophil during the anaphase supports this view, although he regards the erythrophil character of the threads as due to their nutrition by the cytoplasm. According to this observer, as the daughter-nuclei pass through the various stages of the anaphase, large quantities of nutrient material are taken up into the nucleus, and the latter then gives the erythrophil reaction.

In the division of the nucleus in the basidium of the Hymenomycetes, where no cell-plate is formed and where no necessity exists, therefore, for the accumulation of the nutrient material in the equatorial plane of the nucleus, a part of the nucleolar substance would probably be taken up at once into the nuclear threads, and by means of them be transferred to the daughter-nuclei.

This conclusion appears to me to be more in accord with the facts observed. But a certain quantity of the dissolved nucleolar substance probably escapes into the cytoplasm when the nuclear membrane disappears, and this would be taken up at a later stage into the daughter-nuclei, as is shown by the increase in size of the nucleoli, and by the decrease in the capacity of the protoplasm for taking up stains.

In a recent paper, Zacharias[2] brings forward numerous observations to show that the strongly-marked cyanophil reaction of the nuclear thread is due to nuclein. Rosen[3] has

[1] Ueber das Verhalten des Pollens, &c., Histologische Beiträge, Heft iv, 1892, p. 38. [2] Loc. cit. [3] Loc. cit.

also suggested that those parts of the nucleus which stain blue contain nuclein, whilst those which possess no nuclein stain red.

This seems to me to hold good for resting nuclei generally, but as a test for nuclein in dividing nuclei it fails. It is interesting in connexion with this to note that the nucleolus in the basidium of the Hymenomycetes, when the larger part of its substance has become dissolved, stains blue; and Zacharias points out, in the paper above quoted, that if epidermal cells of *Galanthus nivalis* be placed in digestive fluid, the network of the nucleus and the remains of the nucleolus stain blue after a time. It appears to me, therefore, that both network and nucleolus contain substances which give the cyanophil reaction at certain times. In the resting nucleus the blue staining of the nucleolus is masked by a substance which has the erythrophil reaction, whilst in the stages of division the blue of the threads becomes masked by a substance which also possesses the erythrophil reaction: and as the threads become erythrophil, the erythrophil character of the nucleolus disappears.

Division of the Daughter-Nuclei.

The division of the two daughter-nuclei takes place in the same manner as the primary division already described. The nuclear membrane, however, persists for a very much longer time.

A. stercorarius. When the daughter-nuclei are completely formed, they lie in a transverse plane near the apex of the basidium, and, as already mentioned, take up nearly the whole of its diameter. When about to divide, they first of all elongate transversely along a line passing through the centre of each nucleus. The two nuclei come into close contact, and, as there is no room in the basidium for any very considerable elongation in the transverse direction, further elongation takes place in an obliquely upward direction. The two nuclei thus form an obtuse angle with one another, the apex of which points to the lower end of the basidium. The free ends of the nuclei come into contact with the wall of the

basidium, and the nuclear membrane soon disappears at these points, which may be regarded as the apices of the nuclei. The nuclear network now becomes transformed into a number of deeply stained red chromatin-granules, which accumulate at the apex of each nucleus, in contact with the cytoplasm (Figs. 20 and 21). The nucleoli meanwhile become much smaller in size and lose their capacity for taking up the red stain. The rest of the nuclear membrane begins to disappear, and the protoplasm around the two nuclei, at this stage, again stains more deeply than before, probably owing to the escape of the nuclear sap with the dissolved nucleolar substance.

A nuclear spindle now appears, in contact with each group of chromatin-granules. In favourable specimens they can be very distinctly seen (Fig. 22), and at each pole of the spindle a deeply stained granule (centrosome?) is visible. The latter are well seen in Fig. 22, in which the two drawings represent the same basidium at two different levels to show the dividing daughter-nuclei. The very much reduced nucleoli are still visible.

The division of the two groups of chromatin-granules now takes place. The two daughter-groups produced in each case, separate along lines which are parallel to each other, in a transverse plane, near the apex of the basidium. After traversing an angle of about 45° they stop and are then in close contact with the wall of the latter. At this stage, a superficial glance at a transverse section of a basidium shows these separated groups as small deeply stained masses, about equidistant from one another, quite close to the wall of the basidium, and between them the remains of the two spindle-figures (Fig. 23). The faintly stained remains of the nucleoli can still be seen and, on focussing a little deeper, the faintly stained, indistinct portions of the two old nuclear membranes.

A. muscarius. The formation of the equatorial plates in the daughter-nuclei of this species differs slightly from that of *A. stercorarius.* The outline of each nucleus becomes very irregular, and as the chromatic elements are formed they take

up a position on that side of the nucleus nearest to the wall of the basidium and for a short time after their appearance here, a few delicate faintly stained blue threads can be seen traversing the cavity of the nucleus (Fig. 45). These soon disappear however, as well as the nuclear membranes, so that the nuclear elements come to lie, at an early stage, quite freely in the cytoplasm (Fig. 46). The nucleolus, meanwhile becomes much smaller in size and loses its capacity for taking up the red stain. The chromatin-granules forming the equatorial plate in each nucleus are arranged more or less regularly in a ring, which appears perfectly homogeneous under a low power of the microscope. The arrangement recalls that seen in the equatorial plate of the higher plants. The formation of the spindle and the separation of the chromatic elements takes place as in *A. stercorarius.*

The chromatic elements, in both species, as soon as they reach the poles of the spindle, fuse together and present a more or less homogeneous appearance. Then the old nucleoli disappear and soon afterwards a nuclear membrane is formed around each daughter-nucleus. Out of this fused mass of chromatic elements, a nuclear-network becomes gradually differentiated which stains blue like that in the parent nucleus, and the remainder of the chromatic substance appears to become transformed into the nucleolus (Figs. 24 and 47).

The four daughter-nuclei increase in size until they take up nearly the whole of the diameter of the basidium. They arrange themselves in a transverse plane near its apex, and transverse sections through this region of the basidium, at this stage, show the four nuclei very clearly.

The four nuclei now begin to move towards the base of the basidium, and at the same time the sterigmata begin to form as four small projections near the apex (Figs. 25–27). At the base of the basidium the nuclei, which have hitherto been spherical in outline, become more irregular and come into such close contact with one another that it becomes difficult to observe their outlines at all (Figs. 27 and 28). Finally they

appear as if fused together into a homogeneous mass, in which, however, the four nucleoli can still be made out (Fig. 28). Whether this is a true fusion or not I could not satisfactorily determine. That some change takes place is seen in the fact that the network of the nuclei in this condition becomes more deeply stained and thicker than before and the nuclei themselves appear to become contracted.

While this apparent fusion is going on, the spores begin to form at the apex of the sterigmata, and before they are fully formed, the four nuclei begin to separate from each other again (Fig. 29). As this takes place, the nuclei are seen to be extremely irregular in outline and to possess what appears to be a very thick nuclear membrane. This apparent thickness of the nuclear membrane is due, however, so far as I can make out, to the irregularly coiled thick nuclear threads which have taken up a position at the periphery of the nucleus, and when seen in optical section present the appearance of a thick membrane surrounding the nucleus. The ordinary nuclear network, in fact, seems to have disappeared with the exception of a few very slightly stained delicate threads which traverse the cavity of the nucleus. The nucleolus in each case is in close contact with the thick peripheral thread at this stage. As the nuclei separate still further from each other, they gradually expand and assume a spherical outline. Then they begin to move upwards towards the apex of the basidium, and, as this takes place, they gradually regain their former structure and soon present the appearance of normal nuclei with the same staining reactions as they originally possessed. It is difficult to understand the meaning of this curious phenomenon. The fact that, at the moment when the nuclei begin to separate from each other, they present a very much contracted appearance, leads me to suppose that there is no real nuclear fusion but that the production of this confused irregular mass at the base of the basidium is due to the contraction of the nuclei and subsequent alteration in some way or other of the nuclear network. It may be an effect produced at a certain stage by the action of reagents used in the hardening and preparation

of the specimens, owing to some change in the resistance of the basidium to contraction consequent upon the passage of a considerable quantity of protoplasm into the spores which might also affect the nuclei. Or it may be that the nuclei take up this basal position in order to facilitate the entry of protoplasm into the spores, and their apparent fusion in this position may be simply an incidental phenomenon consequent on their being brought into such close contact with each other in the narrow basal portion of the basidium. But these are only suggestions and perhaps other observers may be able to explain the phenonemon more clearly.

The protoplasm of the basidium now becomes less dense owing to the transference of a large quantity of it into the spores which during this time have been increasing very much in size. The nuclei at the same time reach the apex of the basidium and so arrange themselves that one nucleus lies at the base of each of the sterigmata (Fig. 30). This does not take place in *A. stercorarius* until the spores have reached their full size and have become surrounded with a thick brown wall, but in *A. muscarius* it takes place before the spores have become fully developed and while the wall of the latter is yet very thin. The structure of the nuclei is at this stage quite normal. Each consists of a nucleolus which stains dark purple, a nuclear network which stains light blue, and a nuclear membrane which stains nearly the same colour as the nucleolus. They are best seen in transverse sections of the basidium. Soon a change appears in each nucleus, preparatory to its entrance into the spore; the outline becomes irregular and the nuclear membrane more indistinct; at the same time the nuclear network loses its definite appearance and becomes a somewhat homogeneous mass difficult to distinguish from the protoplasm immediately surrounding it; the nucleolus becomes smaller, so much so that it is difficult to differentiate it from the refringent protoplasmic knots which are found at this stage in the basidium. Then, in some way which I have not been able to make out clearly, the nucleus makes its way through the opening of the sterigma into the spore, where it immediately

divides into two, probably by a process of karyokinesis similar to that observed in the basidium, but as I have not been able to make out these points as clearly as I should like, I will reserve the discussion of these observations for a future paper.

In conclusion it may be useful to summarize briefly the chief facts made out in this investigation.

Summary of Results.

1. The young basidia of *Agaricus stercorarius* and *A. muscarius* contain a single nucleus formed by the fusion of two or more pre-existing nuclei.

2. The structure of the nucleus is similar to that in the higher plants; it possesses a nuclear membrane, nucleolus, and granular network. On staining with carmine and nigrosin the network becomes blue, the nucleolus a deep reddish purple.

3. The division of the nucleus is karyokinetic, resembling, generally, that which takes place in the higher plants, but with slight differences of detail. The chromatin-network breaks up into segments, which accumulate at one side of the nucleus. The nucleolus begins to dissolve, and at the same time the chromatic elements begin to stain more deeply than before. The nucleolus does not disappear entirely until the division is nearly complete. A spindle-figure is formed in connexion with the chromatic elements; the latter divide into two groups, which pass along the threads to the poles of the spindle.

4. The chromatic elements fuse together at the poles of the spindle; a new nuclear membrane appears around each daughter-nucleus, and a new nucleolus and nuclear network become differentiated.

5. The daughter-nuclei divide in the same manner as the parent nucleus.

6. The four nuclei thus produced pass at once to the base of the basidium and come into such close contact as to appear fused together. After a time they separate again, pass to the apex of the basidium and place themselves immediately at the base of the sterigmata.

7. The nuclei undergo a change previous to their entry into the spores. They become smaller; the outline and network become indistinct, and hardly distinguishable from the surrounding protoplasm.

8. The actual entry of the nuclei into the spores was not observed.

9. The observations on the colour-reactions of the nuclei in the various stages of division seem to point to the conclusion that a portion of the dissolved nucleolar substance is taken up into the chromatic elements, as fast as the nucleolus becomes dissolved.

EXPLANATION OF THE FIGURES IN PLATES XXIV, XXV, AND XXVI.

Illustrating Mr. Wager's paper on Nuclear Division in the Hymenomycetes.

Agaricus (*Stropharia*) *stercorarius.*

All the figures have been drawn by means of the Camera lucida.

Figures 1–8, 12–21, 23, 24 and 26–30, with Zeiss's apochromatic 2 mm. 1·3 ap. and ocular 18; figures 9–11 and 22 and 25 with Zeiss's apo. 2 mm. 1·4 aper. ocular 18.

Fig. 1. Young basidium with two nuclei, just before fusion.

Fig. 2. Young basidium showing the two nuclei in close contact with each other.

Fig. 3. Nuclei beginning to fuse together.

Fig. 4. Later stage of fusion, the nuclear membrane has disappeared at that point where the nuclei are in contact.

Fig. 5. Later stage, complete fusion, but the two nucleoli are still distinct.

Fig. 6. Later stage, complete fusion of all parts of the two nuclei, including the nucleoli. At this stage the protoplasm is very dense and deeply stained; vacuoles are generally absent. The nuclear network colours deep blue, the nucleolus dark purple or bluish red.

Fig. 7. Basidium with nucleus in the first stage of division. The nuclear thread is breaking up, and the nuclear membrane has disappeared at the upper end of the nucleus.

Fig. 8. Later stage of division. The chromosomes are grouped together at the apex of the nucleus, and now stain deeply a bright red. The nucleolus is smaller in size, and stains light blue. The basal portion of the nuclear membrane still persists.

Fig. 9. Basidium with equatorial plate, or group, of chromosomes and

spindle-figure. At the poles of the spindle are two deeply stained dots (centrosomes?). The nucleolus is still visible, but is much less prominent than before.

Fig. 10. Transverse section of basidium in same stage of development as Fig. 9.

Fig. 11. Basidium with chromosomes, which are beginning to separate into two groups. The nucleolus is still visible, but is very small and faintly coloured.

Fig. 12. Transverse section of basidium at the stage figured in 11.

Fig. 13. Later stage in the separation of the two groups of chromosomes. The basidium was slightly oblique; the lower part was out of focus.

Fig. 14. Stage in which the chromosomes, having reached the poles of the spindle, begin to fuse together.

Fig. 15. Fusion of chromosomes at the poles of the spindle into bright red, irregular masses, around which the new nuclear membranes form.

Fig. 16. Transverse section of basidium, at slightly later stage than that figured in 15.

Fig. 17. Basidium with two daughter-nuclei just formed. The nuclear membrane is distinctly visible, but not the nuclear network.

Fig. 18. Transverse section of basidium, with two daughter-nuclei, at a slightly earlier stage perhaps than Fig. 17.

Fig. 19. Basidium with two completely formed daughter-nuclei. They have now the same structure as the parent nucleus.

Fig. 20. Basidium with the two daughter-nuclei just beginning to undergo changes previous to division. The chromosomes are shown, beginning to accumulate at the apex of each nucleus.

Fig. 21. Oblique section of a basidium, showing one of the daughter-nuclei, with a group of deeply stained bright red chromosomes.

Fig. 22. Basidium, drawn at two different levels to show the two dividing daughter-nuclei. In each can be seen a spindle-figure with deeply stained dots at each pole.

Fig. 23. Transverse section of a basidium, at a later stage than Fig. 22. Complete separation of the chromosomes has taken place. The nucleoli can still be made out, but are very small.

Fig. 24. Basidium with four completely formed nuclei, just after the division of the two daughter-nuclei has taken place.

Fig. 25. Basidium showing sterigmata just beginning to develop.

Fig. 26. Slightly later stage than Fig. 25. The four nuclei have made their way towards the base of the basidium, previously to their coalescence with each other.

Fig. 27. Stage in which the four nuclei are shown combining together.

Fig. 28. Appearance of the four nuclei at a slightly later stage. They are now in such close combination with one another, that the outlines of the individual nuclei cannot be made out.

Fig. 29. Basidium, with a young spore attached to one of the sterigmata (the others having fallen off), showing the nuclei beginning to separate again. They are very irregular in outline and the nuclear membrane appears very thick.

Fig. 30. Basidium at a stage when the spores are fully formed. The nuclei are found at the bases of the sterigmata, ready to make their way into the spores. The structure of the nuclei at this stage appears to be quite normal.

Amanita muscarius.

Figs. 31, 32, 38–43, 45 and 46 Zeiss's apo. 2 mm. 1·4 aper. and ocular 18.

Figs. 33–37, 44, 47 and 48 Zeiss's apo. 1·5 mm. 1·3 aper. and ocular 18.

Fig. 31. Young basidium with three nuclei.

Fig. 32. Young basidium with two nuclei.

Fig. 33. Young basidium with two nuclei, more highly magnified than Fig. 32. The chromatin-granules in the nuclear network are distinctly visible. These granules stain deep blue, the nucleolus deep bluish red.

Fig. 34. Basidium, with single large nucleus produced by fusion of the two or three original nuclei. This nucleus clearly shows the same structure as the nuclei of the higher plants. The nucleolus colours an intense bluish red, the granules in the network, deep blue.

Fig. 35. Basidium with nucleus, in which the nuclear thread, or threads, is broken up into a number of short, slightly curved, rods.

Fig. 36. Basidium with nucleus in a later stage of development than Fig. 35. The outline of the nucleus is irregular; the threads are shorter and thicker and more sharply curved, in some cases forming a somewhat homogeneous mass, coloured deep blue.

Fig. 37. Later stage. The nuclear membrane has almost completely disappeared. At the upper end of the nucleus is a small number, six to eight, of deeply stained, short rods or granules coloured bluish red, and between these a few delicate lightly stained blue threads are to be seen.

Fig. 38. Stage in which the nuclear membrane has entirely disappeared. The chromosomes form now an equatorial plate, and are stained bright reddish blue. The nucleolus is visible, but is very small and stains light blue.

Fig. 39. Basidium with nuclear spindle visible; the chromosomes are just beginning to separate into two groups. The nucleolus still visible, but very small. The chromosomes at this stage are stained bright red.

Fig. 40. Nearly same stage as Fig. 39, but the nucleolus has disappeared.

Fig. 41. Slightly later stage than Fig. 39. Eight chromosomes can be made out.

Fig. 42. Later stage. The chromosomes have now divided into two groups; the remains of the spindle-threads can be seen connecting the two.

Fig. 43. Basidium with two daughter-nuclei just formed. The chromatin-masses still stain bright red.

Fig. 44. Basidium with two completely formed daughter-nuclei. Each nucleus possesses the same structure as the original parent nucleus, and stains in the same way.

Fig. 45. Basidium with daughter-nuclei just previously to division of the latter. Each nucleus possesses a group of deeply stained red granules (chromosomes), and a number of light blue threads. The nuclear membrane is very irregular in outline, and the nucleolus has become smaller.

Fig. 46. Basidium showing the equatorial groups of chromosomes of the daughter-nuclei. The nucleolus is visible in one case; in the other it was out of focus, so was not drawn.

Fig. 47. Basidium with four nuclei produced by division. The structure of each nucleus is the same as that of the original single nucleus of the basidium.

Fig. 48. Basidium just before the formation of spores. The nuclei form a closely adpressed mass at the base of the basidium.

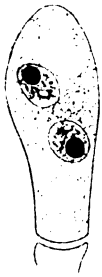


Fig. 1.

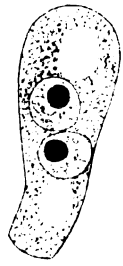
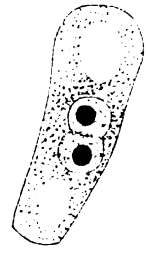


Fig. 2.



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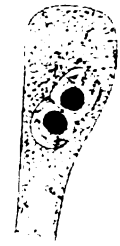

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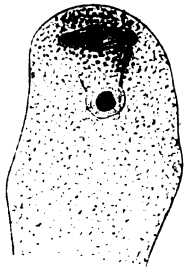

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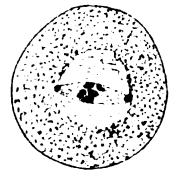

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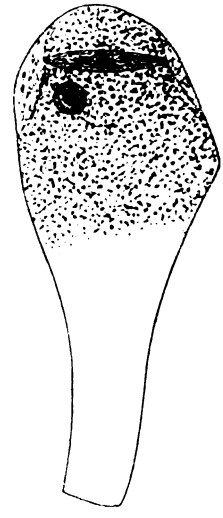

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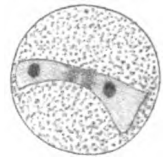

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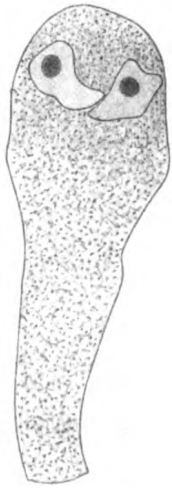

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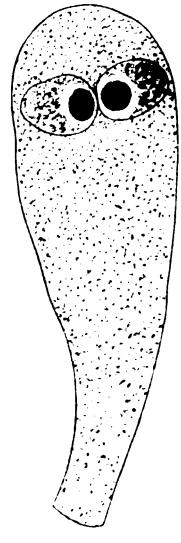

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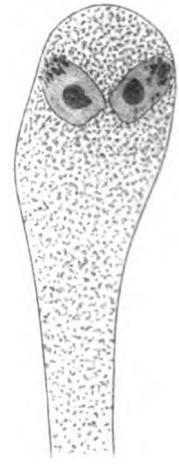

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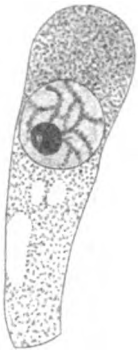

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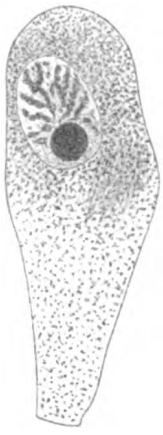

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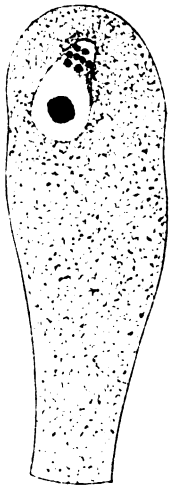

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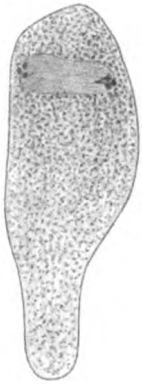

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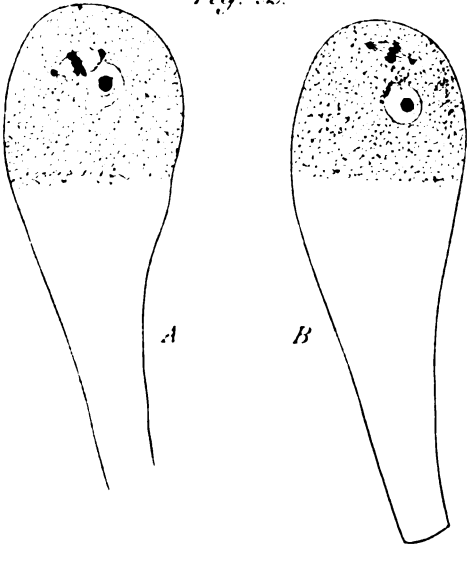

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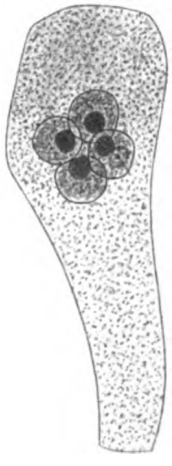

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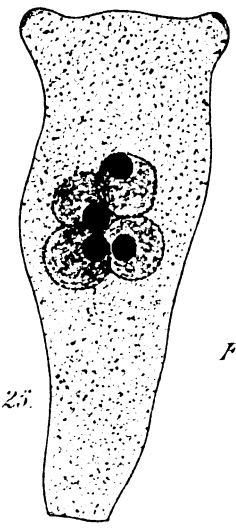

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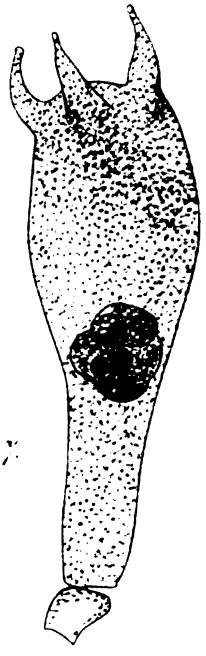

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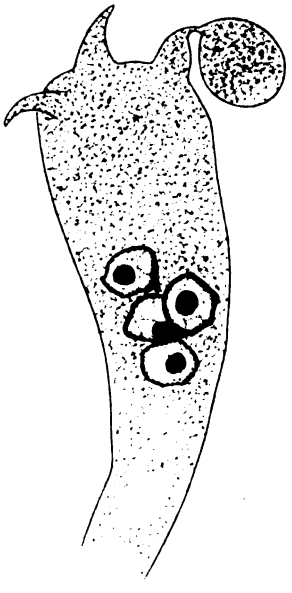

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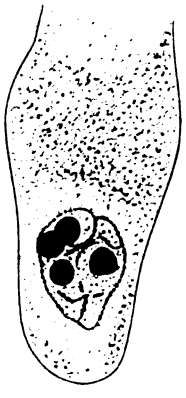

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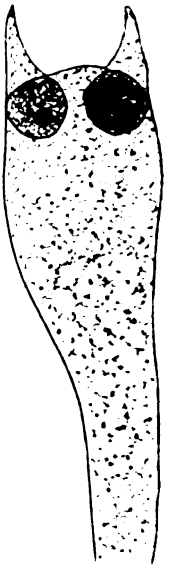

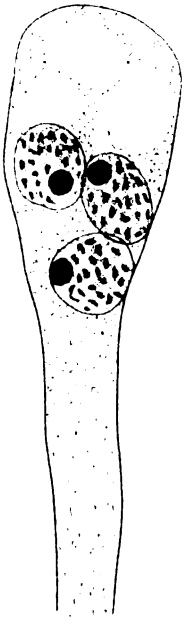

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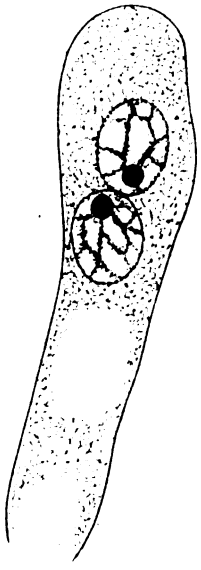

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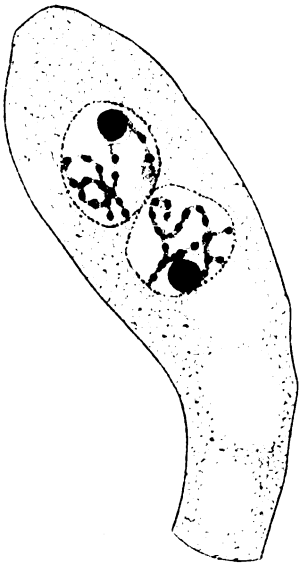

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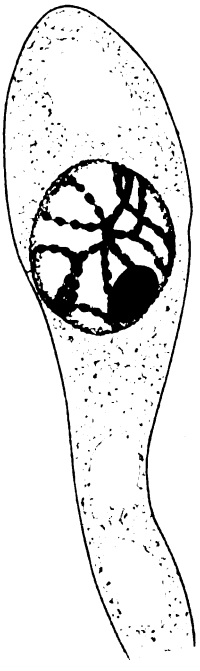

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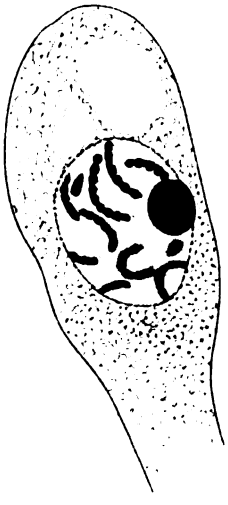

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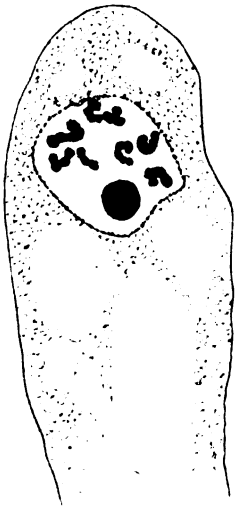

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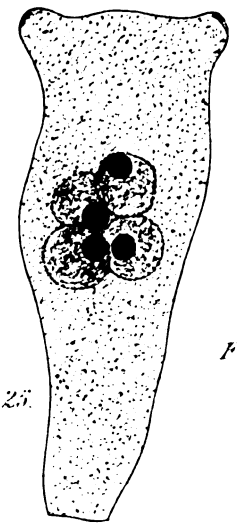

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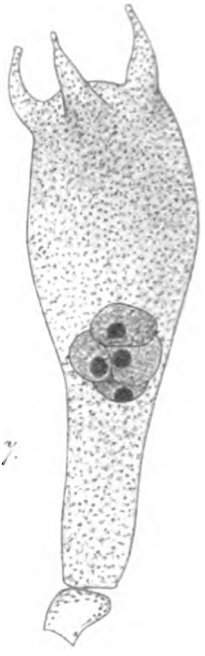

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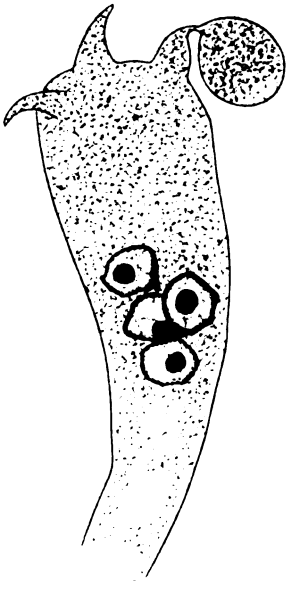

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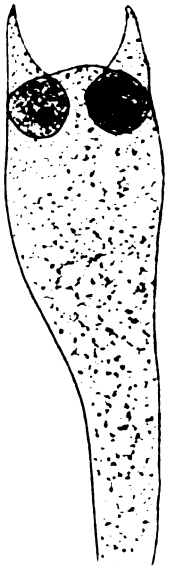

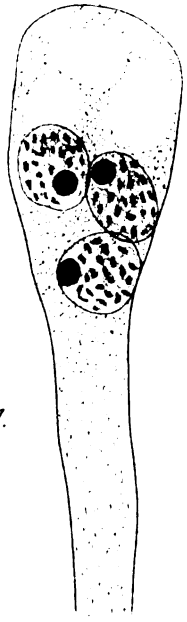

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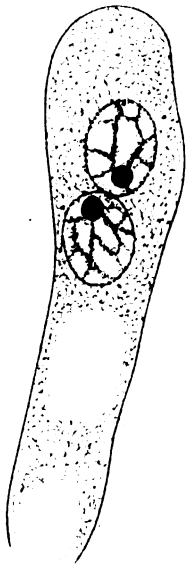

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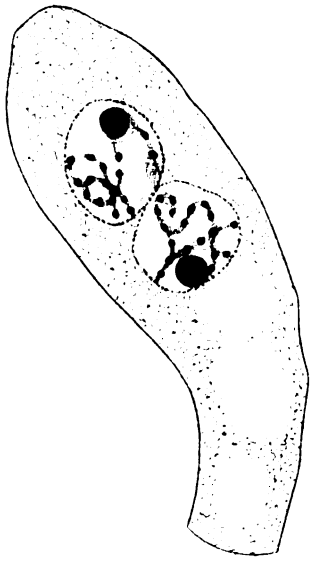

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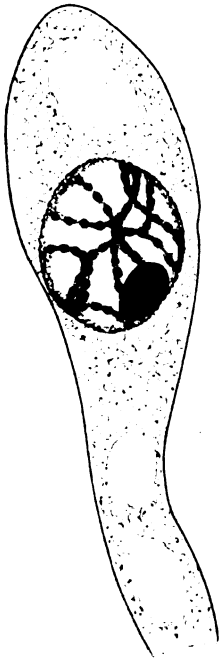

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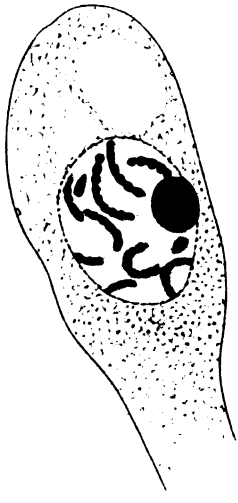

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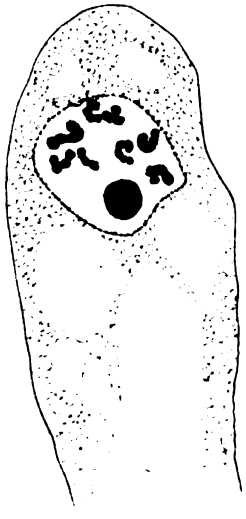

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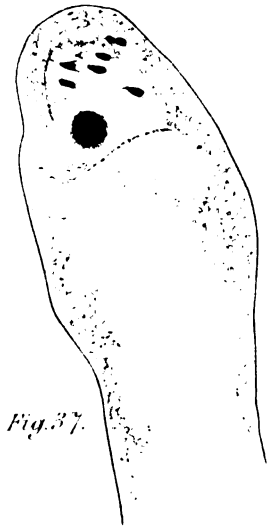

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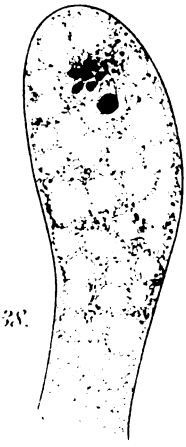

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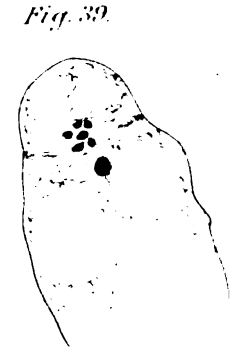

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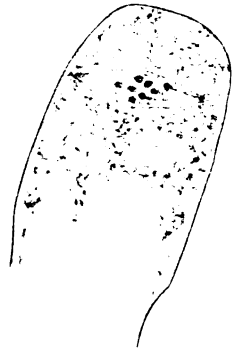

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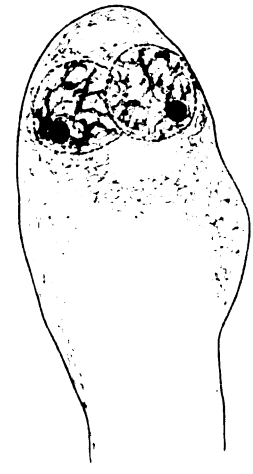

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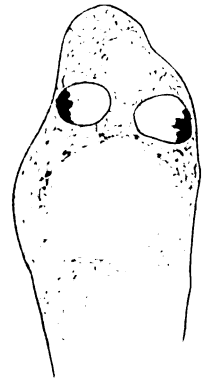

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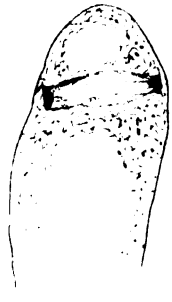

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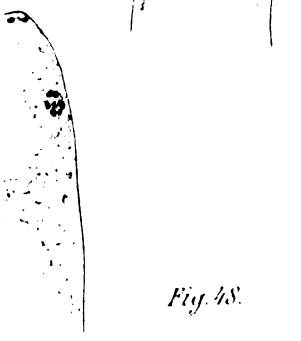

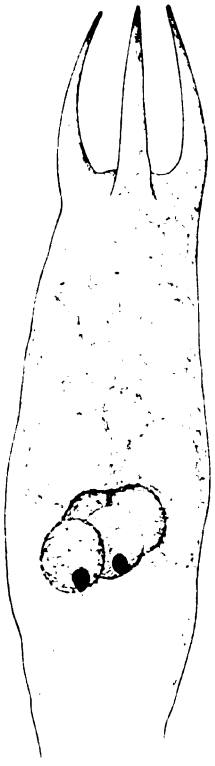

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:chari, Mass.; a Fun- : of the Sugar-cane.

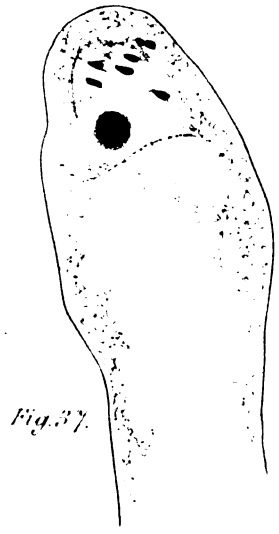
SEE,

um, Royal Gardens, Kew.

XXVII.

ars a considerable amount of n addressed to the Royal gar-cane disease in the West Superintendent of the Royal the first to call attention to e disease to be caused by erial sent being either dry or uld not be corroborated by mained unsettled until the gar-canes, illustrating every ovell, Superintendent, Botani-, Barbados. Each cane was the supposed cause of disease, *Xyleborus perforans*, Wall.); *lis*, Fabr.); 'fungus'; and in hese were described as being as requested by the Director a thorough examination of

December, 1893.]



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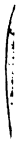


Fig. 38.

Fig. 41.

Fig. 43.

Fig. 42.

On Trichosphaeria Sacchari, Mass.; a Fungus causing a Disease of the Sugar-cane.

BY

G. MASSEE,

Principal Assistant, Herbarium, Royal Gardens, Kew

With Plate XXVII.

DURING the past three years a considerable amount of correspondence has been addressed to the Royal Gardens, Kew, relating to a sugar-cane disease in the West Indies. Mr. J. H. Hart, F.G.S., Superintendent of the Royal Botanic Gardens, Trinidad, was the first to call attention to the subject, and considered the disease to be caused by a fungus, but owing to the material sent being either dry or in alcohol, this supposition could not be corroborated by cultures; and the question remained unsettled until the receipt of eighteen selected sugar-canes, illustrating every stage of the disease, from Mr. Bovell, Superintendent, Botanical Station, Dodd's Reformatory, Barbados. Each cane was accompanied by a description of the supposed cause of disease, as, attacked by 'shot-borer' (*Xyleborus perforans*, Wall.); 'moth-borer' (*Diatraea saccharalis*, Fabr.); 'fungus'; and in some instances two or all of these were described as being present on the same cane. I was requested by the Director of the Royal Gardens, to make a thorough examination of

[Annals of Botany, Vol. VII. No. XXVIII. December, 1893.]

the canes, with the view of throwing light upon the nature of the disease.

A preliminary report was published in the 'Kew Bulletin' for July, 1893, and in this the macroconidia and microconidia of the present paper were considered as the first stage in the life-cycle of the fungus, and the *Melanconium* as the second stage, followed by the ascigerous condition. Extended cultures and experiments show that this sequence is not correct. The *Melanconium* condition is the first stage, its conidia producing macroconidia, which in turn yield the highest or ascigerous condition.

It is probable that the fungus called *Trullula Sacchari*, E. & E., is identical with the macroconidia of the fungus under consideration, which does not accord with any known species. I have accordingly named it *Trichosphaeria Sacchari*.

Macrosporium stage.

All inoculation experiments were made on sugar-canes growing in the Lily House (No. 15) at Kew, and all cultures unless otherwise stated, were made in a solution of sugar-cane, prepared by soaking small pieces of cane in water; after remaining for two days the liquid was filtered and sterilized by intermittent boiling.

Conidia of the present form, obtained from West Indian canes, germinated in two days in a hanging drop. The conidia are continuous at maturity, but immediately preceding germination, the contents separate at the middle into two equal portions leaving a clear space: but I was unable to demonstrate the formation of a transverse septum, although it is well known that the spores of some fungi that are continuous at maturity become distinctly septate and many-celled on germination, as in many of the Tremellineae, each cell giving origin to a germ-tube. The same thing occurs in the promycelium of *Puccinia* where the apical portion is divided into three or four cells by transverse septa, each cell producing a sporidium or secondary spore. The significance of this partitioning of the protoplasm into separate portions, in each

of which a nucleus can in many instances be demonstrated, is not understood, but in the case of a segmented spore all the germ-tubes usually grow at about the same rate and form equally vigorous hyphae; whereas in unicellular spores several germ-tubes are often produced that grow at an almost equal rate for some time, one finally continuing to grow, whereas the remainder become arrested and die after having apparently expended so much energy for no specific purpose. In *Oedocephalum roseum*, Cke., the spores are large, unicellular, and contain a well-defined nucleus which disappears at the commencement of germination; several germ-tubes are produced, one of which continues to grow, the remainder soon dying: in the persistent hypha a nucleus can be readily demonstrated, whereas no such structure can be shown to exist in the hyphae or germ-tubes that perish prematurely. Is the presence of one or more nuclei necessary for the continuous growth of a germ-tube?

The conidia generally form two germ-tubes, one from each localized portion of protoplasm, which are about 2 μ thick, aseptate, at least for a considerable time, and soon give off lateral branches of equal diameter to the parent hypha (Fig. 5).

In flask-cultures the conidia behave as already described, but development proceeds much further.

Four conidia were placed in each of three flasks containing an equal quantity of sugar-cane solution; the flasks were placed on a shelf fixed over a hot-water coil in the laboratory the temperature averaging 75° F., the extremes being within 5° of that point. At the end of five days the liquid in each of the flasks presented an opalescent appearance, which examination of the contents proved to be due to very delicate, much branched hyphae equally diffused throughout the liquid. The nutrient solution had a slightly acid reaction, and the addition of potassic hydrate, amounting to one per cent. for the whole solution, completely checked further growth of the mycelium. Examination of the contents of a second flask after eight days' growth showed numerous filaments of mycelium measur-

ing up to 8 μ diameter and full of brilliant, fine grained, homogeneous protoplasm. These thick hyphae originated as lateral branches from the delicate hyphae first produced by the conidia. No trace of fusion of hyphae was observed in any of the numerous cultures examined, when grown in sugar-cane solution, whereas in every other nutrient solution used, conjugation of the hyphae, brought about by the curving of a branch as described by Ward [1], were abundant. I have frequently observed in cultures of other fungi that conjugation of the hyphae is more abundant when grown in a nutrient solution differing materially from the one most closely allied to the normal condition of life of the species, i. e. a solution of the host or substratum. The addition of a one per cent. solution of cupric sulphate killed the mycelium in flask number 2.

After twelve days' growth the contents of the third flask assumed a dark olive colour and the entire surface of the mycelium at the level of the solution presented the appearance of an olive-coloured, dense, velvety pile. The velvety appearance proved to be due to the presence of closely packed, erect, dark olive conidiophores growing out into the air, each bearing at its apex a single chain of reddish-brown conidia—to be spoken of in future as *microconidia.* The dark colour of the mass of mycelium immersed in the fluid was found to be due to immense numbers of large conidia arranged in chains and springing from the tips of the thick hyphae previously described. These last will be distinguished as *macroconidia,* and, along with the microconidia, will be described in detail later.

Small portions of sugar-cane, containing hyphae of the *Macrosporium,* were placed in the nutrient solution, and in twelve days the liquid was crowded with mycelium bearing both forms of conidia mentioned above. As illustrating the vitality of the hyphae, it may be mentioned that thirteen weeks after the diseased canes were received at Kew, a small

[1] *A lily-disease*; Ann. Bot., Vol. II, p. 329 (1888).

portion of one containing hyphae was placed in the nutrient solution, and in three weeks conidia were present and the mycelium was as abundant as when grown from fresh material.

As already stated, the external evidence—in the form of fruit—of the parasite is confined to the basal half of the cane, and the very delicate vegetative hyphae are difficult to observe in the tissues. Nevertheless when internal portions of a diseased cane, taken from near the apex, are placed in a nutrient solution, care being taken to guard against the accidental introduction of conidia, the rapid growth of hyphae and eventual formation of macroconidia prove the presence of hyphae throughout the length of the cane. By this method the presence of the parasite was demonstrated near the apex of every cane sent for examination; the accuracy of this method was proved by microscopic preparations made from portions of the pieces used for the flask-cultures.

When a cane is first attacked by the fungus, the hyphae are only present in the elements of the bundles; very soon, however, the hyphae extend to the fundamental tissue, at first travelling along the line of the middle lamellae and giving off numerous branches that enter the cells; this is, so far as I have been able to observe, accomplished by passing through a pit in the wall: this point is, however, more readily seen in the case of the thick mycelium developed at a later stage, and will be afterwards described.

The presence of actively growing hyphae in the tissues in the basal or older part of a cane is always indicated by a more or less clear red colour; this, however, is not so marked in the upper and younger part of the cane when hyphae are present. This difference suggests the presence of some substance capable of being acted on by the fungus in the lower and older part of the cane and absent from the upper and younger portion. Similar red blotches are produced by a fungus causing a sugar-cane disease in Java, which has in consequence received the name of Rood Snot[1]. The red

[1] *Het Rood Snot*; Dr. Went, Mededeel. van het Proefstation West-Java, pp. 1–18, (1893) 2 pl.

colouring-matter is most abundant in the elements composing the bundles, which show up as bright red streaks when the parasite has but recently commenced its work; at a later stage the fundamental tissue also becomes tinged. The colouring-matter appears under the form of patches of variable size attached to the inside of the cell-wall and is soluble in alcohol.

As no indication of disease due to the *Trichosphaeria* had been observed on the sugar-cane cultivated at Kew, it is assumed that the results to be recorded were entirely due to inoculation from material obtained from the diseased canes received from Barbados.

Expt. I.—A sugar-cane six feet high and one and a half nch diameter at the base was experimented upon. *Melanconium*-conidia were placed upon the base of an old leaf-sheath, the leaf having fallen away: after twenty days the *Melanconium*-fruit was fully developed, the long black filaments of conidia oozing out through minute cracks in the cuticle about half an inch above the node, and from the point of inoculation. At the same time as the above experiment was performed, a small portion of diseased cane containing hyphae of the *Melanconium*-stage was introduced into a slit made in a cane; this experiment resulted in the appearance of mature fruit bursting out from the cane after twenty-two days. The cane was cut down ten days after the last-mentioned experiment, and on being split open it was found that at the point where inoculation was performed by wounding the cane, the mycelium had produced the large macroconidia in the decaying tissue; no macroconidia were present at the point where infection took place through a dead leaf-base. This agrees with what has been observed in canes from the West Indies, macroconidia being met with only in those cases where the tissue was decayed.

Expt. II.—*Melanconium*-conidia were placed on moistened patches of young living leaves of sugar-cane, some of the patches being first carefully washed to remove the bloom on the surface of the leaf, others not being so treated. After

twelve days there was no sign of any disturbance of the tissues due to the entrance of a parasite, although some of the spores had germinated on the surface. Repeated experiments with conidia on an unbroken surface of young stems and leaves also gave negative results; hence it may be concluded that conidia of the *Melanconium* phase can only obtain an entrance into the cane through a broken surface, and judging from an examination of diseased canes, the most vulnerable points are the bases of dead leaves low down on the cane. The bases of broken-off lateral shoots and rootlets, and the burrows of the 'shot-borer' also favour the entrance of the fungus.

When the vegetative hyphae of the *Melanconium* have become abundant in the tissues of the sugar-cane, they form dense wefts immediately below the epidermis and between two bundles; the weft commences to grow in a very compact manner and eventually forms a dark-coloured parenchymatous cushion or stroma composed of small polygonal cells about 5 μ in diameter; from 1–3 loculi are present in the mature stroma; the cells lining the loculi each send out one delicate spine-like hyaline sterigma or pedicel which in turn bears a narrowly cylindrical, uniseptate, pale brown, straight or very slightly curved conidium measuring 14–15 × 3·5–4 μ (Fig. 4). When mature the stromata vary from 1–2 mm. in length, and owing to development taking place in the direction of the epidermis, the latter eventually splits and the black surface of the stroma protrudes through the crack. When mature the conidia ooze out of the cavity in which they were formed through a minute pore or ostiolum at its apex, and being held together by mucus derived from the deliquescence of the external portion of the exospore, remain in the form of minute black, more or less curled filaments, to 2–3 mm. long which become rigid when exposed to the air (Figs. 2 and 3).

The *Melanconium* form of fruit was not reproduced in any of the flask-cultures containing the nutrient solution only, but the characteristic stroma and conidia—both colourless—were

produced on a piece of healthy Kew cane that was placed in a flask containing mycelium produced by *Melanconium*-conidia.

Macroconidia.

As already stated, pure cultures have proved that the hyphae developed from *Melanconinm*-conidia eventually give origin to stout lateral branches which in turn bear apical chains of macroconidia. These stout branches of hyphae vary from 5–8 μ in diameter and give off numerous lateral branches at short intervals. When growing in a nutrient solution, some of these lateral branches grow to a great length before they branch, and have numerous transverse septa; finally, a considerable number of short lateral branches appear at intervals, these are also transversely septate, and usually more or less curled or contorted and lobed, and undergo no further modification. An examination of the hyphae of this phase of the fungus growing in the tissues of a cane shows that these septate hyphae are vegetative in function, the main branch following the middle lamella, and giving off short lateral branches which enter the cells and act as haustoria.

The short, sparsely septate or aseptate branches are filled with dense, finely granular protoplasm which contains no vacuoles until the apex of the hypha becomes slightly clavate, when the protoplasm becomes frothy and vacuolated. The apex of the hypha continues to swell until it is about twice the diameter of the hypha, when it gives origin to a moniliform chain of macroconidia produced in basipetal succession as follows (Fig. 8). If transverse septa are originally present they are absorbed previously to the formation of conidia. When the tip of the hypha has become sufficiently enlarged, the apex of the cell-wall swells up and finally disappears, the contained protoplasm slowly protruding through the cavity thus formed (Fig. 11). When the protoplasm has extended for a distance of 10–12 μ beyond the open apex of the mother-cell, a transverse septum appears in the portion

just within the mother-cell; this septum, however, is confined to the protoplasmic contents and not connected with the wall of the mother-cell. The segmented apical portion of protoplasm continues to grow out of the mother-cell, accompanied by a simultaneous constriction at the septum and rounding off of the apical portion of protoplasm, which eventually projects beyond the sheathing open apex of the mother-cell as a spherical conidium, attached by a constricted neck to the protoplasm included within the mother-cell. In one instance the formation of a conidium, from the moment of deliquescence of the apex of the wall of the mother-cell to the complete formation of the exserted globose conidium, occupied forty-six minutes. When quite young, the protoplasm of the conidium presents the appearance of a coarse, irregular network, due to numerous vacuoles; these however soon disappear, and three or four smaller persistent vacuoles take their place. When developed under normal conditions in the cane, two permanent vacuoles are usually present, one at each pole. Chlor-iodide of zinc demonstrated the presence of a delicate cell-wall of pure cellulose on the exposed part of the developing conidium, the colour indicating this dying away at the point still contained within the mother-cell; When first formed the wall of the conidium is colourless; in about twelve hours it becomes tinged with clear olive-green; in twenty-four hours it becomes sooty-brown, and finally opaque blackish-brown. Succeeding conidia are formed in a manner similar to the first, but differ in being elliptical or barrel-shaped with truncate ends, whereas the first formed terminal conidium is always spherical, measuring 24–26 μ in diameter, the barrel-shaped ones averaging 18–20 x 12 μ. In cultures fifty conidia are often present in a chain, and in one instance sixty-seven were counted (Fig. 8). When developed in a cane, the conidia are smaller, the terminal one measuring 20 μ diam., the others 16–19 x 10 μ, and as a rule not more than twenty conidia are present in the chain.

From the description given, it will be observed that the macroconidia are formed in a manner unusual amongst fungi,

but which resembles in many points the formation of hormogonia in some of the lower Algae, as described and figured by Bornet and Thuret[1]; in this instance, however, the concatenate cells forming a hormogonium are completely formed within the mother-cell, escaping from its ruptured apex at maturity.

Amongst Fungi, I have observed an exactly similar mode of conidial development in *Milowia nivea*, Mass.[2], and *Sporoschisma mirabile*, B. & Br.[3]; it is also evident from the description and figures given by Halstead and Fairchild[4] of *Ceratocystis fimbriata*, Ell. & Hals., a fungus causing black rot in the sweet potato, that the two forms of conidia present, correspond in structure and origin with the macro- and microconidia of *Trichosphaeria Sacchari*, and although the ascigerous stage of the *Ceratocystis* has not yet been discovered, the close agreement between the conidia in the two species suggests affinity.

When the conidia-bearing branches of the *Trichosphaeria* become swollen, the cell-wall becomes bright blue at the apex when treated with a solution of iodine, resembling in this respect the asci of many of the Discomycetes and other ascigerous fungi. No trace of nuclei, as morphologically understood, could be detected in the mother-cell nor in the conidia during any stage of development; it is true that a number of points in the mother-cell, and one in each conidium, became coloured with those reagents that differentiate nuclei, but it appears to me rather doubtful in all such cases as to whether such apparently structureless particles are the equivalents in function of true nuclei, whatever that function may be.

Inoculation of healthy canes with macroconidia yielded the following results.

Expt. I. Placed on the basal part of the upper surface of

[1] Notes Algologiques, Plates III, XXX, XXXVII, and XXXVIII.
[2] Journ. Roy. Micr. Soc., v. 4, ser. 11, p. 841, Pl. XII.
[3] Berk., Intr. Crypt. Bot., Fig. 74 *a*.
[4] Journ. Mycol., Vol. vii, p. 1, Pl. I–III.

a very young leaf; in five days the infected area became deep red, and in fourteen days a dense pile of conidiophores appeared on the surface bearing microconidia; hyphae were abundant in the tissues, but internal macroconidia were not formed. Other experiments proved that the conidia were developed much earlier when the surface was first washed with soap and water. No inoculation took place when conidia were sown on full grown but still vigorous leaves.

Microscopic examination showed that the germ-tubes of the conidia pierced the cuticle of the young leaf, and did not enter through the stomata.

Expt. II. A lateral shoot was broken off close to the stem and conidia sown on the broken surface. In fourteen days the wounded surface was covered with microconidia, but although hyphae had penetrated for some distance into the main stem, no macroconidia were present.

Expt. III. A notch two inches long was cut into a stout cane near the base, and a quantity of the interior of the cane cut out, making a large cavity into which two conidia lying on a cover-glass were placed, the cover-glass being rubbed against the wounded surface of the cane to insure the contact of the conidia. The external slit was closed up by tying lead-foil round the stem. In twenty-two days the broken internal surface presented a bright red coloration, and an abundance of mature chains of macroconidia were present in the wounded portion of the tissue. This experiment, along with others, proves that macroconidia are only formed in the interior of a cane, and when the tissue is disorganized (Fig. 6).

Expt. IV. Conidia were sown on the ragged portion of a leaf-base close to the main stem, and a thin slice of the surface of the cane was cut away half-an-inch above the point of infection. In nineteen days microconidia appeared on the surface of the wounded portion, clearly proving that infection can take place by conidia falling on dead portions that are still in contact with the living cane, as leaf-bases, broken roots, lateral branches, &c.; and as these organs originate close to the nodes, the points where the disease first shows

itself in diseased canes, it seems probable that the fungus usually effects an entrance at these points.

The hyphae belonging to the macroconidium-stage very frequently follow the middle lamellae for some distance, giving off lateral branches that pass into the cells through a pit in the wall, the cavity of which does not become at all enlarged, the closing membrane alone being dissolved. When the tip of a hyphae extending along the surface of the wall passes over a pit, a minute papilla projects into the cavity and grows through the pit as a delicate strand about 1·5 μ thick; immediately on emerging at the opposite side of the wall, the delicate filament assumes a spherical form 5–6 μ diameter, and then continues elongating as a hypha of equal thickness with the one from which it originated on the opposite side of the wall. The hyphae do not cling to the wall inside the cell: some branches remain in the cell and utilize its contents; others grow straight across until they come in contact with the opposite wall, and if the growing point does not happen to alight on a pit at once, the hypha follows the surface of the wall, searching for one, through which it passes by a narrow neck into an adjoining cell; or very frequently follows the middle lamella. By this method the hyphae travel quickly through the tissues. In some preparations the hyphae in following the middle lamella are seen to take a sinuous course, as if searching for the sparsely scattered pits, and not unfrequently five or six branches enter into, and grow across a cell, all originating from a single hypha growing along the line of the middle lamella (Figs. 12, 13). Very good preparations showing the hyphae passing through the cell-wall, as described above, result from first soaking the sections for an hour in a five per cent. solution of potassic hydrate, washing thoroughly in water to get rid of the potash, and afterwards staining with Bismarck brown or picro-nigrosin. Very old hyphae are naturally of a pale brown colour, and require no staining.

Microconidia.

This form of reproduction is a modification of the one last described, developing from the same hyphae, and owing its structural peculiarities to exposure to light and air during growth; thus illustrating from an unexpected quarter, a general rule amongst groups of Fungi showing a transition from a subterranean to an aerial condition. The various stages are clearly developed in sequence in the Gastromycetes, where, from the subterranean Hymenogastreae with an undifferentiated indehiscent peridium, very large spores, and without any arrangement for spore-diffusion, we pass to the above-ground Lycoperdeae with a differentiated peridium, capillitium, and small spores, the modifications being for the purpose of facilitating spore-dispersion by wind; and finally in the Phalloideae we find the mature sporophore elevated into the air at the top of a long stem-like receptacle, usually furnished with bright colours and a penetrating odour, for the purpose of attracting insects by whose agency the very minute spores are dispersed.

In the present instance, the advance made in the general structure of the microconidia over the macroconidia tends in the direction of favouring the dispersion of the conidia by wind; the entire fructification is developed in the air, the conidiophores are elongated, and the conidia are comparatively minute (Fig. 7, *c*, *d*.)

Preceding the formation of microconidia, the hyphae in the tissues concentrate at those points where the surface of the cane has been recently wounded and form a dense weft; the hyphae becoming thinner as they approach the surface. From this weft the aerial conidiophores originate in immense numbers, forming a velvety pile on the surface of the wounded portion. The conidiophores when mature are of a pale grey colour, sparingly septate, and vary from 150–220 μ in length, become swollen to a breadth of 12–16 μ at a short distance from the base, and gradually taper to the apex where they are about 6 μ in diameter. The conidia are developed in

a chain at the ruptured apex of the conidiophore in a manner precisely similar to the macroconidia already described; they are elliptic-oblong with truncate ends, of a clear pale reddish brown colour at maturity, and measure on an average 10–11 × 6 μ. The number of conidia in a chain rarely exceed ten, and the terminal one is shaped like the rest, and not spherical as in the macroconidia (Fig. 10).

Expt. I. Microconidia placed in a cavity made in a cane produced macroconidia in the interior of the cavity and microconidia at its surface.

Ascigerous Stage.

Two mature perithecia were found on a much decayed portion of one of the canes received from Barbados; they sprang from a point that had previously borne a crop of microconidia, and were surrounded by old collapsed conidiophores, the conidia having disappeared. Although the evidence in favour of a genetic connexion between the perithecia found on the cane and the microconidia with which they were associated was strong, yet it could not be accepted as conclusive; and it was not until similar perithecia were accidentally discovered on the surface of the material contained in one of the flask cultures, that this supposition was proved to be correct. The flask in question was one of which the contents were not required during the investigation, and contained a dense mass of hyphae produced from a macroconidium. The submerged portion of the hyphae was black from a copious development of macroconidia, while the surface was covered with a dense pile of conidiophores bearing microconidia. One day this flask was accidentally broken, and out of curiosity a portion of the contents, taken from the surface bearing microconidia, was placed under the microscope, when something much resembling a very young perithecium was seen. Further search revealed the presence of two young perithecia, almost colourless and without fruit, but bearing the long, characteristic bristle-like septate hyphae, present on the mature

perithecia found on the decayed cane; two examples of the initial stage of a perithecium were also found. The culture was placed under favourable conditions for the further formation of perithecia, but unfortunately soon became covered with *Penicillium* and other growths, and gave no further results.

The earliest stage in the formation of a perithecium observed consisted of the fundamental coil, forming the apex of a short lateral branch 4 μ thick; the coiled portion was provided with several transverse septa; there was no trace of an archicarp—Woronin's hypha—within the coil.

The mature perithecium is broadly ovate and furnished with a minute pore or ostiolum at the apex, blackish brown in colour, and sparsely clothed with long, septate, tapering, dark brown, rigid hairs; numerous brown, septate hyphae spring from the basal portion of the perithecium, forming a densely interwoven subiculum (Fig. 17); asci cylindrical, narrowed at the base into a curved pedicel; spores 8, obliquely uniseriate, hyaline, continuous, elliptic-oblong, ends obtuse, $8-9 \times 4\ \mu$; paraphyses absent (Fig. 19).

Expt. I.—Spores obtained from the mature perithecia found on the decayed cane germinated tardily in a hanging drop of sugar-cane extract: these were placed respectively on the base of a broken-off lateral shoot, and on the remains of a dead leaf-base of sugar-cane, but unfortunately no result followed; hence it is not known whether the ascigerous fruit gives origin to the *Melanconium* stage as is presumably the case.

Experiments proved that macroconidia of the *Trichosphaeria*, sown on very young leaves of bamboo, germinated and formed hyphae in the tissues; the red coloration, so characteristic of the presence of the parasite in the sugar-cane, was not present.

Finally, it was observed that when a cane was attacked by both *Trichosphaeria* and the beetle known as the 'shot-borer' (*Xyleborus perforans*, Wall.), the numerous pellets of excrementa present in the burrows of the latter were found

to consist to a large extent of fragments of mycelium and conidia belonging to the *Trichosphaeria*. These pellets when placed in a nutrient solution, gave origin to vigorous hyphae, produced from the conidia present, and also from the masticated fragments of hyphae (Fig. 21). It is known that the female beetle usually visits several canes, boring each in succession hence: it is highly probable that healthy canes may, through the agency of the beetle, become inoculated with the fungus.

Summary.

Experiments made with healthy sugar-cane grown at Kew demonstrate conclusively that the fungus called *Trichosphaeria Sacchari* can effect an entrance into healthy canes, quite independently of the agency of the 'shot-borer' or 'moth-borer.'

Although a true parasite, in the sense of destroying perfectly healthy, living tissues, the fungus almost invariably commences as a saprophyte, the conidia germinating on the remains of dead leaf-bases, scars formed by broken lateral branches, roots, &c., the hyphae afterwards passing into the living, uninjured tissue of the cane; and judging from the fact that the disease is always most mature at the lower and older portion of the cane, it is evident that the fungus effects an entry by the means indicated. The cultures described also prove that the fungus can pass through the entire cycle of its development as a saprophyte.

It is to be regretted that the entire cycle was not completed by reproducing the *Melanconium* stage from ascospores. It is possible that the highest, or ascigerous phase, is on the wane, as proved to be the case in other species of Fungi that have become decided parasites. The early saprophytic condition is a decided advantage in the present instance, bearing in mind the silicious cuticle of the sugar-cane. The external development of microconidia I am inclined to consider as a comparatively modern development from the less differentiated internal macroconidia; the object in view being

to effect a rapid extension of the species by wind-borne conidia.

As to immediate protective measures; all diseased plants should be burnt at once, and not allowed to accumulate as rubbish. No portion of a diseased cane should be used for propagation, neither should apparently healthy canes be used that are obtained from infected areas. It is almost certain that the great amount of litter strewed on the ground in the cane-plantations acts as a nurse to the fungus: hence it would be a great advantage, wherever practicable, to plant the ground previously occupied by cane, with other crops for a year or two, first burning all the litter. If the above precautions were persistently carried out, the fungus would be kept well in check, and no longer constitute a grievous disease.

Finally, the endeavour to eradicate the disease should be general. It would be a waste of energy on the part of one planter to carry out all the necessary precautions in the neighbourhood of a neglected plantation. The entire evidence points to the conclusion that the disappearance of the disease will follow prompt and universal action on the part of those concerned.

EXPLANATION OF FIGURES IN PLATE XXVII.

Illustrating Mr. Massee's paper on *Trichosphaeria Sacchari*.

Fig. 1. Portion of a cane showing the *Melanconium*-stage of the fungus in the young condition. Nat. size.

Fig. 2. Portion of a cane showing the *Melanconium*-stage in the mature condition; the projecting threads consist of myriads of conidia held together by a mucilaginous substance. Nat. size.

Fig. 3. Section through a mature *Melanconium*-pustule showing the stroma with two conceptacles, from one of which a filament, *a*, of conidia has been expelled. × 200.

Fig. 4. Portion of wall of a conceptacle in the stroma of *Melanconium* showing the origin of the conidia. × 650.

Fig. 5. Conidia of *Melanconium*, two of which are germinating. ×650.

Fig. 6. Portion of a cane split down the centre, showing, *a*, the large internal black mass of macroconidia; and *b*, the external microconidia forming a minutely velvety, blackish patch. Nat. size.

Fig 7. Portion of the tissue of a cane showing the formation of, *a*, *a'* internal macroconidia; *b*, external microconidia, borne in chains *c*, at the tips of long dark-coloured hyphae or conidiophores, *d*. ×650.

Fig. 8. Portion of hypha producing macroconidia. Drawn from a pure culture grown in a sterilized solution of cane-sugar and produced from a macroconidium. Specimens grown under such circumstances are much larger than the same form when produced normally in the tissues of the cane; some of the chains contain sixty-seven conidia, and the apical globose conidium is very large and distinct. ×650.

Fig. 9. Macroconidia germinating; *a*, intercalary conidia; *b*, terminal globose conidium. The conidia were obtained from a pure culture. ×650.

Fig. 10. Microconidia germinating. The conidia were obtained from a pure culture. ×650.

Fig. 11. Tips of hyphae giving origin to macroconidia; *a*, showing the cell-wall at the apex of the hypha just commencing to deliquesce for the purpose of allowing the protrusion of the protoplasm which forms the terminal conidium of the chain; *b*, the same further advanced, the protoplasm having grown out of the ruptured sheath and formed a cell-wall of its own. ×1000.

Figs. 12, 13. Mycelium belonging to the macroconidium-stage present in cells of the fundamental tissue of the cane. In passing through the cell-walls, *a*, *a'*, the pits in the wall are always used, a narrower portion of the hypha passing through and at once assuming the normal diameter on the opposite side. ×650.

Fig. 14. A branch of mycelium that has sent two branches into a living cell; the branches have developed into lobed haustoria. ×650.

Fig. 15. A short lateral branch of mycelium showing at its tip the fundamental coil, *a*, preceding the formation of a perithecium. ×650.

Fig. 16. A very young perithecium still attached to the parent hypha, *a*. ×650.

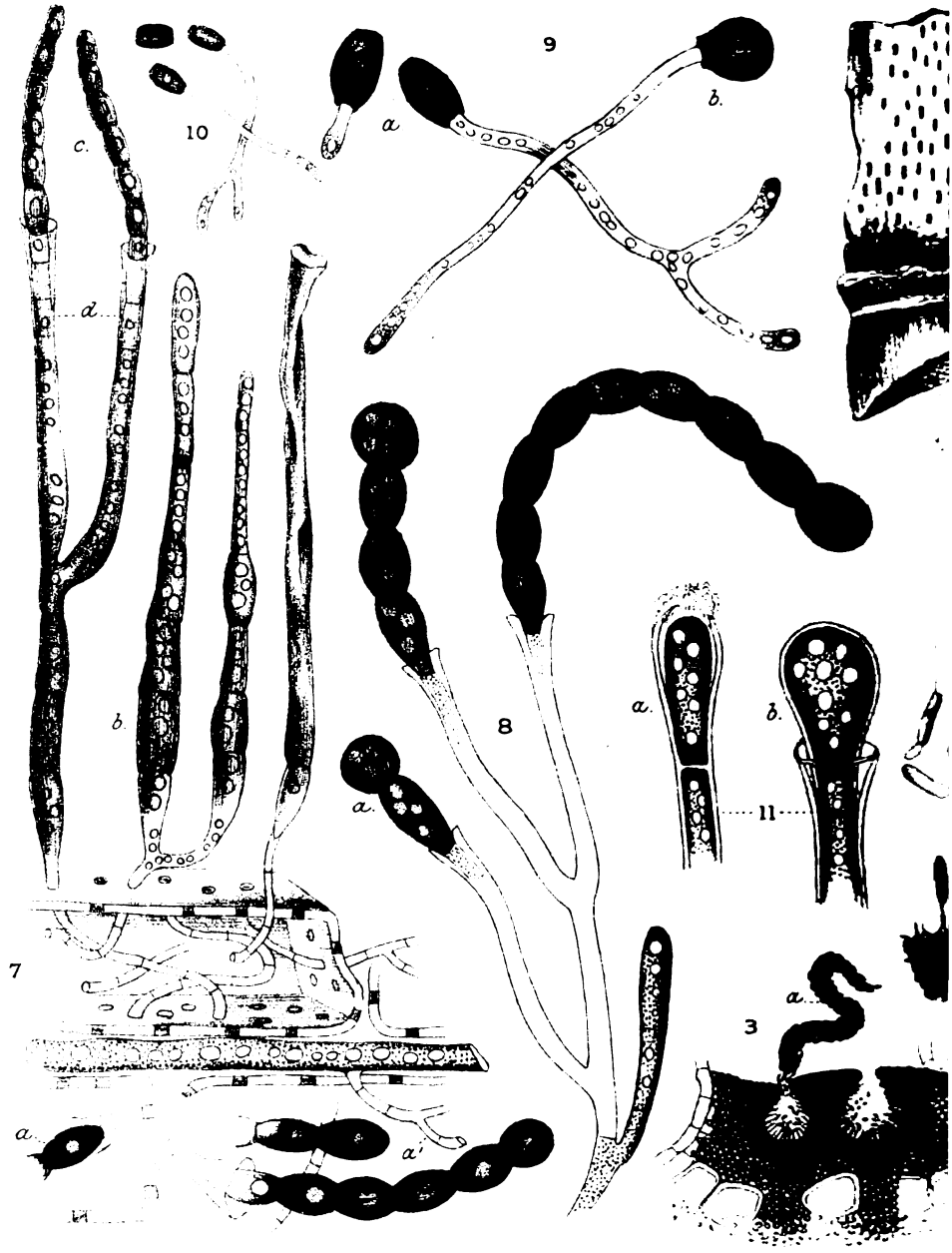
Fig. 17. A mature perithecium. ×350.

Fig. 18. One of the large coloured hairs from the external wall of the perithecium. ×500.

Fig. 19. Asci from the perithecium in various stages of development; one is mature and containing eight spores. ×650.

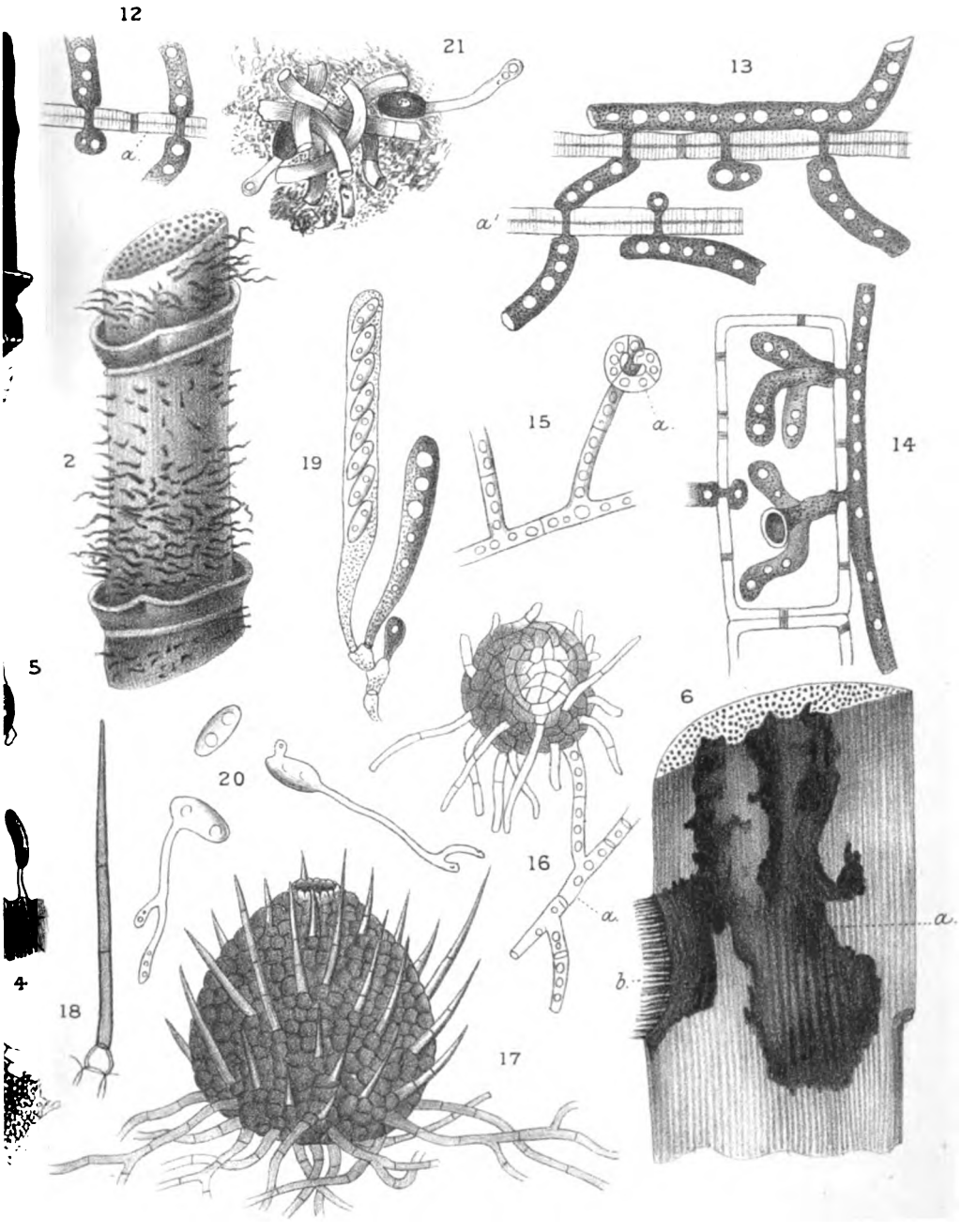
Fig. 20. Ascospores liberated from the ascus (Fig. 19). Two of the spores are germinating. ×750.

Fig. 21. Portion of a pellet of excrementa belonging to the cane-boring beetle, consisting of broken up mycelium and macroconidia, the latter are germinating. ×650.



Massee del.

MASSEE. — TRICHOSPHÆRIA.



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